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THE IMPORTANCE OF MICROPALÆONTOLOGICAL STUDIES IN INDIA*

By Prof. L. Rama Rau, F.A.Sc.

In opening the Proceedings of this meeting, let me at the outset convey on behalf of the Biological Section, our hearty felicitations to our President Sir C. V. Raman on the happy occasion of the celebration of his 60th birthday, and wish him many more years of active service in the cause of Science. Though a physicist, many of his recent researches have a distinct 'biological' aspect; and after seeing his mineral collection in the 'Crystallographic' exhibition yesterday, we geologists feel that he is almost one of us! I wish also to extend a hearty welcome to all the Fellows and Delegates who have gathered here and thank them for their kind co-operation in the successful organisation of this meeting. Having been called upon to preside and deliver an Opening Address to this distinguished gathering, I wish to avail myself of this opportunity to draw your earnest attention to a very important and fascinating field of palæontological research for which there are splendid opportunities in India, *viz.*, the 'Study of Microfossils'. For quite a long time now, the value of fossils—to the geologist on the stratigraphical side, and to the biologist, on the evolutionary side—has been fully recognised, and the subject of Palæontology has come to occupy an important and honoured place among the Biological Sciences. But most of this palæontological work relates to the bigger and more easily recognisable fossils found in the rocks; it is only recently that we have realised the fact that very minute organic remains—both plant and animal—are often found in rocks, which become visible only when they are examined under the microscope. These 'microfossils' are just as interesting and important as the bigger ones; in fact, in some cases, they are even more so. Their study should therefore form an important part of all palæontological investigations, whether approached from the geological side, or the biological.

I will now proceed to give a brief review of such micropalæontological work done in India within recent years, to indicate the great interest and importance of this line of research. It is a field of work in which all Natural Scientists—geologists, botanists and zoologists—could participate in a co-operative effort; and if in the course of this short Address, I succeed in stimulating, in some of you at least, an active interest in this subject, I shall be quite happy.

Casual references to micropalæontological work in India have been made now and then in some of the geological papers. In view of the special importance of the study of microfossils to the oil-field geologists, in their

* Presidential Address delivered at the Scientific Meeting in Section B of the Indian Academy of Sciences held on 27-12-1948 at Bangalore.

work of detailed stratigraphical classification and correlation, the Oil Companies, as for instance, the B.O.C. have done a considerable amount of micropalæontological work; but unfortunately, the results of their investigations are not published, and are therefore not readily available. My own interest in the subject started about 20 years ago, in connection with the microscopic study of some of the Cretaceous rocks of South India, in many of which I found the abundant occurrence of small foraminifers. With a view to describe and identify these microfossils, hundreds of sections were being examined; and it was found that several of them contained, in addition to the foraminifers, plenty of fossil algæ. This discovery was reported in 1931; and in view of the fact that this occurrence of abundant fossil algæ was the first of its kind to be reported from India, and that this algal flora was particularly rich and varied, their study immediately attracted our whole time attention, and an elaborate Memoir dealing with the fossil algæ from the Niniyur Group was published in the year 1936 in collaboration with Prof. Julius Pia of Vienna. It is gratifying to find that this soon opened out an extensive field of research on upper Cretaceous and lower Tertiary algæ in India, of special interest alike to the stratigraphical geologist and the palæobotanist. Messrs. S. R. Narayana Rao and K. Sripada Rao (who were still recently my colleagues and are now occupying the Chairs in Geology in the Lucknow and Nagpur Universities respectively) have pursued this line of work and published quite a number of very valuable papers dealing with fossil algæ from various parts of India. But I wish to point out that what has been done so far, is only touching the fringe of this problem. The scope and possibilities for the study of fossil algæ are indeed very vast, and it is a most interesting field of research. I hope that many more workers will develop an interest in this line of work in India, and thus contribute to the advancement of our knowledge of fossil algæ, in general.

While these fossil algæ demanded most of our attention in the earlier years, the study of the formainifers was not altogether forgotten. In view of the special importance of this group in the classification and correlation of Cretaceous and Tertiary strata, the study of these fossils was pursued in right earnest; and this led to the discovery in 1939 of an *Eocene* bed in the Pondicherry area overlying, and probably in continuation of, the Cretaceous series. Mr. S. R. Narayana Rao who continued these investigations for more than ten years now, has published several valuable papers dealing with the foraminifers from the Cretaceous rocks of South India, Tertiary beds of Surat and Broach, Kampa Dzong beds of Tibet, and the Yaw Stage of Burma. Important contributions in this field have also been made recently by other workers, notably Nuttall, Pinfold, and Davies, working on the

Eocene rocks of North Western India. As in the case of fossil algæ, here again, it must be pointed out that what has been done so far in this branch of micropalæontology is just a beginning; the entire field is very vast and almost limitless. It now requires an enthusiastic band of workers to engage themselves actively in this field of research; and the results are bound to be exceedingly interesting and important.

A most interesting contribution in micropalæontology was made in the year 1939, when the Lucknow School of Palæobotany, under the inspiring leadership of Prof. Birbal Sahni, reported the discovery of numerous ' spores ' and ' pollen grains ' in the beds intimately associated with the glacial deposits of India and other parts of Gondwanaland. In the course of his Presidential Address to this section of the Academy in 1938, Prof. Sahni himself gave an account of these studies and the important light they throw on the interesting problem of the relation of the Glossopteris Flora with the Gondwana glaciation; and also its bearing on several other geological and palæogeographical aspects in the study of that ancient continent.

The most recent investigation that has brought out in a striking manner the importance of micropalæontological studies in tackling geological problems is that connected with Prof. Sahni's discovery, in 1943, of numerous ' microfossils ' in the Saline Series of the Punjab Salt-Range, and their bearing on the intriguing and highly controversial problem of the age of the Saline Series. It is hardly possible for me here to go into the details of this 60-year old controversy, nor is it necessary for me to do so, since I am sure that most of you are familiar with the main arguments under discussion. The present conclusion based on the evidence of the newly discovered microfossils is entirely in favour of the Tertiary age; and the serious doubts entertained in some quarters regarding the truly *in situ* nature of these fossils must be dispelled by the fact that these microfossils are found not only in the 'Salt beds ' but also in the associated dolomites and oil shales. The verdict of these fossils thus becomes supreme, and their testimony in favour of the Tertiary age is obviously irresistible. This work on the Saline Series is an outstanding example of how a study of minute microfossils may throw light on some of the biggest and most controversial problems.

In this brief review, I have referred to just 4 of the recent lines of research in the field of micropalæontology, but I think I have said enough to impress upon you the value and importance of micropalæontological studies, for which there is such ample scope in India. It is very likely that many of the rock formations which we are now considering as ' unfossiliferous ' because we do not see any obvious fossils in them, might on suitable treatment and examination under the microscope, reveal the presence of microfossils in

a smaller or larger measure, throwing some light on the age of the containing beds, which would be important not only in itself, but might also help us in interpreting the tectonic complications and decipher the true disposition and mutual relationship of beds in a disturbed area. As I have said elsewhere—" When once we begin to deal with the field relationships of fossiliferous beds whose ages can be determined on the internal palæontological evidence, the verdict of the fossils becomes supreme, and we are no longer at the mercy of tectonic interpretations for the age determination of the concerned beds. On the other hand, the known ages of the beds must help us in deciding the acceptability or otherwise, of any field interpretation."

Apart from the examination of microfossils and their identification under the microscope such as those described above, I venture to suggest that another line of micropalæontological investigation appears possible and likely to yield fruitful results; and that is, the detailed study, under the microscope, of the structure of certain hard parts of animals which alone are preserved in the fossil condition—especially for instance the shells of Brachiopods and Molluscs. It is well known that these shells are composed mostly of crystalline grains and fibres of minerals like calcite and aragonite, with a definite arrangement and structure. If this could be investigated under the microscope with reference to their optical character and orientation in different genera and species of shells, and some correlation is established between their shell structure and their generic and specific affinities, it would be a great step forward; for it may then be possible, by such studies to identify a form, even if only a few fragments of its shell are found, as is often the case, in the fossil condition. Especially in cases where entire forms are altogether absent and such fragments, which alone may be found, are now being considered as practically useless, such optical studies of the shell structure conducted on a large scale may enable us to recognise the form to which they belong and thus make them useful for stratigraphical purposes. It may be that we might have to invoke even the aid of X-Rays in such studies. In fact, some years ago, a short paper was published in our Academy Proceedings on the " X-Ray Analysis of the structure of Iridescent Shells " belonging to a few living forms, and the observations made in this paper are of great interest to the biologists also. Would it not be possible to do similar work, suitably modified if necessary, in the case of fossil shell structures, and see if it will not lead to results useful to the palæontologist ?

The subject of micropalæontology offers a most interesting and fruitful field of research, with immense possibilities in India, and it is earnestly to be hoped that it will soon attract a team of enthusiastic workers all over the country, especially in our Universities and Research Institutes where alone conditions are favourable for the pursuit of such pure scientific research.

EXOBASIDIUM FROM SOUTH INDIA

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Received April 30, 1948
(Communicated by N. L. Dutt, Esq. M.Sc., F.A.Sc.)

The occurrence of the genus *Exobasidium* in India was observed as early as 1895 (McRae, 1910) when 'blister blight' of tea was investigated in upper Assam and the causal fungus was identified as *Exobasidium vexans* Massee. Since then six more species of this genus have been recorded in this country (Butler and Bisby, 1931) occurring on six genera of host plants. But all of them have been noticed only in North India especially in the Himalayan region.

In August, 1946 a severe epiphytotic of blister blight broke out on tea for the first time in South India. During the exceptionally wet and prolonged rainy weather experienced during that year the disease spread rapidly over most of the tea estates in Travancore, Anamalais, and the Nilgiris. Since the occurrence of this epiphytotic, interest in this genus was aroused and attempts were made to find out whether this fungus was prevalent in South India on any other host. In early stages tea planters were anxious to know how the disease appeared so suddenly and whether the parasite could have passed on to tea from some other wild host growing in the forests in the neighbourhood of tea estates. Numerous specimens of various plants showing hypertrophy or any sort of fungal growth were forwarded for identification. But so far no other host has been discovered for *Exobasidium vexans* in South India. However other species of *Exobasidium* have been collected during visits to some of these estates and these are described in this article. Three of these are new species and one is a new record for India.

1. *Exobasidium vexans* Massee.

Subba Rao, M. K., *Blister blight in South India*, 1946, Paper 4, United Planters Association of South India.

McRae, W., *Bull. Agri. Res. Inst. Pusa*, 1910, No. 18.

This parasite is now found in all the tea districts of South India. Its incidence is greatly influenced by the environmental and climatic conditions. Heavy incidence is common during the monsoon months with a decline during the months of January to May.

2. *Exobasidium vaccinii* (Fuck.) Wor.

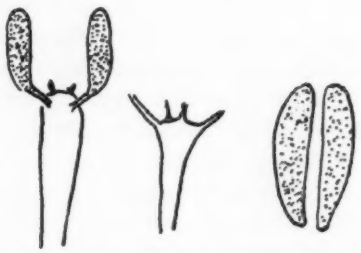
Saccardo, P. A., *Syll. Fung.*, 1888, **6**, 664.

Hotson, J. W., *Phytopath.*, 1927, **17**, 207–16.

Zeller, S. M., *Mycologia*, 1934, **26**, 297.

Spots round or irregular, amphigenous, raised, light to deep pink in colour; hymenium hypophyllous, white, downy; basidia cylindric to clavate, emerging in groups between the epidermal cells, 18–21 × 8–10 μ, normally with four sterigmata; basidiospores hyaline, 1-celled, oblong or slightly curved, 8–15 × 2–4 μ.

On living leaves and twigs of *Vaccinium neilgherrense* W., Naduvattam, 21–5–47 T. S. Ramakrishnan and T. V. Subramanian; Coonoor, 13–10–47, T. S. Ramakrishnan; Chinnakallar (Anamalais) 5–2–1948, T. S. Ramakrishnan and K. Ramakrishnan.



TEXT-FIG. 1. *E. vaccinii*—Basidia and basidiospores (×500)

The diseased leaves can be readily distinguished by the bright pink colour on the upper surface and the white compact fungal growth on the lower. Old spots turn brown and the centre becomes dark and brittle with growth of saprophytic moulds. The young twigs are also infected and these dry up. Basidia are normally 4-spored but sometimes only two or three sterigmata may be present. Witches' brooms or gall formations are not seen.

A number of species of *Exobasidium* have been recorded on *Vaccinium* spp. Judging from the pathological symptoms and the size and characters of the basidia and basidiospores the fungus under study is identified as *E. vaccinii* (Fuck.) Wor. This is the first record of this species from India.

3. *Exobasidium butleri* Syd.

Sydow H. et P. and Butler, E. J., *Ann. Myc.*, 1912, **10**, 279.

Leaves reduced in size, pinkish yellow to bright pink; hymenium spread over the entire lower surface, white to very light pink; basidia club-shaped,

hyaline, usually with four sterigmata but sometimes 3–5 present; basidiospores hyaline, 2-celled, curved, 16 × 4 μ (10–20 × 3–5); secondary spores formed by the germination of the basidiospores, smaller, 1-celled, oblong to cylindric, 6 × 2 μ (3–8 × 1.5–3).

On living leaves of *Rhododendron nilagiricum* Zenk. Ootacamund, 21–5–1947, T. S. Ramakrishnan and T. V. Subramanian.

The infection is apparently perennial and is confined to the young shoots at the apices of the branches. The diseased shoots are conspicuous by their pink colour and most of the leaves on the shoot exhibit the fungal growth on the lower surface. Later the leaves dry up and are shed. The basidiospores are 1-celled when attached to the basidium but later a distinct septum is formed in the middle and the spore is slightly constricted at the septum. Older spores become strongly vacuolated and often give an impression of being more than 2-celled. Numerous secondary spores formed by the germination of the basidiospores can be recognised. These are smaller than the basidiospores and always 1-celled. Sydow (1912) has described this fungus on *R. arboreum* from the Himalayas, and the fungus under study agrees with this. This is the first record of this fungus from South India.

TEXT-FIG. 2. *E. butleri*—(*a*) Section of leaf showing fascicles of basidia (×250)
(*b*) Basidium and basi diospores—one germinating (× 400)

4. *Exobasidium nilagiricum*. Ramakrishnan, T. S. and K. sp. nov.

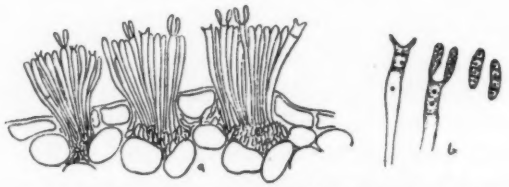
Spots amphigenous, 10–15 mm. diam., round or extensive by coalescence, raised; hymenium hypophyllous, compact, white; basidia long clavate, occasionally septate, hyaline, 52 × 6 μ (32–80 × 4–8), sterigmata 2; basidiospore hyaline, 0–4 spetate, cylindric, straight or slightly curved, 19 × 4.5 μ (12–24 × 4–6).

On living leaves of *Gordonia obtusa* Wall. Naduvattam (Nilgiris), 14–3–1946, (type) T. S. Ramakrishnan and K. Ramakrishnan; Coonoor, 25–2–48, K. Krishna Menon.

Maculæ amphigenæ, orbiculares, sæpe confluentes, 10–15 mm. diam.; hymenio hypophyllo, albido, compacto; basidiis longis, clavatis, interdum

septatis, hyalinis, bisterigmatisis, 52 × 6 μ (32–80 × 4–8); sporis hyalinis, 0–4 spetatis, cylindraceis, rectis vel curvatis, 19 × 4.5 μ (12–24 × 4–6).

In vivis foliis et caulibus *Gordoniæ obtusæ* Wall. Naduvattam (Nilgiris) 14–3–1946, T. S. Ramakrishnan et K. Ramakrishnan (typus) and Coonoor 25–2–1948, K. Krishna Menon.



TEXT-FIG. 3. *E. nilagiricum*—(*a*) Section of leaf with fascicles of basidia (× 200) (*b*) Basidia and basidiospores (×300)

This fungus has been observed from different parts of the province, namely Naduvattam, Ootacamund, Coonoor and Kodaikanal. A herbarium specimen of *G. obtusa* collected by the late Sir A. Bourne from Kodaikanal in 1897 is now available in the Madras Herbarium, at the Agricultural College, Coimbatore. On one of the leaves a spot caused by this fungus is present and in sections of fragments of the spot the pathogen is clearly visible. Thus it would appear that the fungus under study has been in existence for a long time in the province though it is recognised only now.

The spots on young leaves are pink and slightly raised towards the upper surface with a corresponding depression on the lower surface. The latter is covered with white compact hymenium. The spots are usually circular but the shape changes when two or more spots coalesce or when these develop near the margin. Older spots are brown. Very often dark mouldy growths develop on both sides of the spots when they become old, obliterating the original fungus. The central portions of the spots may become torn and bits of leaf tissue may fall off. The hyphæ are intercellular and permeate the mesophyll of the leaf. The basidia develop in bundles which push aside the epidermal cells. Young twigs are also affected and these dry up presenting a scorched appearance.

Inoculation experiments carried out by Mr. K. Krishna Menon, Assistant Mycologist, Ootacamund, with this fungus on *Gordonia obtusa*, showed that positive infection took place in 14–16 days under humid conditions when spores from infected material in the early stages of infection were used. This fungus did not pass on to tea. Attempts to bring the fungus into culture on agar media failed.

An interesting feature of the fungus is the development of partition walls in the basidia. One or two septa are formed towards the apex of the basidium. Two sterigmata are developed and these bear one basidiospore each. Septation of the spores is evident only in late stages just before shedding. Conidia were not observed.

Exobasidium monosporum Saw. has been recorded on *Gordonia* sp. from Formosa (Diehl, 1933). The fungus under study produces two spores per basidium and is therefore different from the former in which only one spore is formed from each basidium. The formation of septa in the basidia and the development of a number of cells in the basidiospores coupled with the measurement of basidia and basidiospores differentiate this fungus from other species. Hence it is described as a new species and named *E. nilagiricum* taking into consideration its common occurrence in the Nilgiris.

5. *Exobasidium celtidis* Ramakrishnan, T. S. and K. sp. nov.

Spots amphigenous, circular or irregular; hymenium hypophyllous, white; basidia clavate, hyaline, 4-spored; basidiospores oval or elliptical, hyaline, 1-many septate, sometimes muriform, 12 × 7 μ, (9–15 × 5–9).

On living leaves of *Celtis tetrandra* Roxb., Ootacamund, 12–3–1946, T. S. Ramakrishnan and K. Ramakrishnan (type); Singampatti, 10–9–1947, Sladden.

Maculæ amphigenæ, orbiculares vel irregulares; hymenio hypophyllo, albido; basidiis clavatis, hyalinis, 4-sterigmatisis; sporis ovatis vel ellipticis, hyalinis, 1-pluri septatis, interdum muriformibus, 12 × 7 μ (9–15 × 5–9).

In vivis foliis *Celtidis tetrandræ* Roxb., Ootacamund, 12–3–1946, T. S. Ramakrishnan et K. Ramakrishnan (typus); Singampatti 10–9–1947, Sladden.

TEXT-FIG. 4. *E. celtidis*—Basidia and basidiospores (×500)

The spots are prominent on the lower surface with a thick white growth of the hymenium. The basidia are stout and bear four short sterigmata. The basidiospores are two celled in the early stages with one transverse septum. There is a slight constriction at the septum. When the spores become older they become 4–5-celled with transverse and vertical septa. They germinate readily forming short germ tubes from one or more cells. The spores are rounded at the apex and have a small pointed projection at the base. The contents are granular.

The shape and structure of the spores differ from those of other described species. Further no species of this fungus has been described on this host. This fungus is unique in having large numbers of muriform basidiospores and hence it is described as a new species.

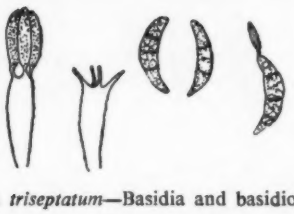
6. *Exobasidium triseptatum.* Ramakrishnan, T. S. and K. sp. nov.

Spots amphigenous, round, 1–3 cm. diam., upper surface convex and lower surface concave; hymenium mostly hypophyllous, compact, white; basidia clavate, hyaline, continuous, sterigmata 4; basidiospores hyaline, curved, 3-septate, 20 × 7 μ (14–28 × 3–8).

On living leaves and twigs of *Vaccinium leschnaultii* W., Ootacamund (Nilgiris), 23–3–1948 (type) T. S. Ramakrishnan and G. Rangaswamy.

Maculæ amphigenæ, orbiculares, 1–3 cm. diam., superficie superiore convexum, inferiore concavum; hymenio sæpe hypophyllo, albido, compacto; basidiis clavatis, hyalinis, continuis, sterigmata 4; sporis hyalinis, curvatis, 3-spetatis, 20 × 7 μ (14–28 × 3–8).

In vivis foliis et rarius caulibus *Vaccinii leschnaultii* W., Ootacamund (Nilgiris) 23–3–1948 (typus) T. S. Ramakrishnan et G. Rangaswamy.



TEXT-FIG. 5. *E. triseptatum*—Basidia and basidiospores (×400)

Prominent raised (concavo-convex) spots are formed on the leaves. The upper surface is either pink or light-green in colour. The hymenium forms a white compact growth on the lower surface. In old spots basidia may be seen on the upper surface also. The basidia develop in closely packed groups and emerge in fascicles pushing aside the epidermal cells. These are broader at the apex with 3–4 prominent sterigmata. The four spores appear clumped at first when the sterigmata are straight but later the latter point outwards and the spores are separated. When fully mature the spores taper towards the base and are fusoid or falcate. Three distinct septa are visible.

Secondary spores which are hyaline and rod-shaped develop in large numbers from the old basidiospores. These originate from the ends or laterally near the septa. Each cell is capable of producing one or more

secondary spores. They measure $6 \times 1 \cdot 5 \mu$ (3–11 × 1–2·5). When scrapings from old spots are examined the secondary spores predominate.

The fungus under study differs in the possession of 4-spored basidia with 3-septate basidiospores from *E. vaccinii*, *E. vaccinii-uliginosi* Bond. and other species recorded on *Vaccinium* spp. Zeller (1934) has described *E. Burtii* on Azalea (*Rhododendron albiflorum*) from America which according to him is intermediate between *E. vaccinii* and *E. vaccinii-uliginosi* in the breadth of the spore but different in type of leaf-spot and in having 3-septate spores. In this species conidia are abundant and the basidiospores are allantoid or ellipsoid and have sometimes longitudinal septa. In the fungus under study conidia were not evident. The spores are different in shape with one end more tapering than the other. Vertical septa are not seen. *E. magnusii* Woron. (Saccardo, 1925) is another species with 1–3 septate basidiospores occurring on *Rhododendron flavus* in Caucasus. But the spores are cylindrical with curved apex. The fungus under study has curved spores which are 3-septate and occurs on *Vaccinium leschnaultii*. On account of these differences it is described as a new species and is named *E. triseptatum*.

This list does not pretend to include all the species of *Exobasidium* present in South India. This genus was not recorded from this region till the sudden outbreak of blister blight on tea in 1946. As a result of this it gained in importance and a preliminary search for other hosts of *E. vexans* has resulted in the discovery of the other species of *Exobasidium* from this area. The absence of previous records of this genus from South India is no indication of its recent introduction as is shown by the presence of *E. nilagiricum* in the herbarium material of *Gordonia* collected as early as 1897.

In conclusion the writers wish to acknowledge their sense of gratitude to the following gentlemen; Mr. K. Krishna Menon, Assistant Mycologist, Ootacamund, for kindly allowing us to use his results of inoculations with *E. nilagiricum* on *Gordonia*, Mr. Sladden of the Bombay-Burmah Trading Co., Singampetti, for the specimen of *Celtis tetrandra*, and the Government Lecturing and Systematic Botanist, Coimbatore, for kindly lending the herbarium specimen of *Gordonia obtusa*.

References

Burt, E. A. .. *Ann. Missouri Bot. Gard.*, 1915, **2**, 627–56.

Butler, E. J. and Bisby, G. R. .. *Fungi of India*, 1931, 93.

Diehl, W. W. .. *Rev. App. Myc.*, 1933, **12**, 698.

Hotson, J. W. .. *Phytopath,*. 1927, **17**, 207–16.

McRae, W. .. *Bull. Agri. Res. Inst. Pusa*, 1910, No. 18.

Saccardo, P. A. .. *Syll. Fung.*, 1888, **6**, 664.

.. *Ibid.*, 1925, **23**, 553–556.

Subba Rao, M. K. .. *Blister Blight of Tea in S. India*, 1946.

.. *Paper* 4, *United Plant. Assoc. S. India.*

Sydow, H. & P. & Butler, E. J. .. *Ann. Myc.*, 1912, **10**, 274–80.

Zeller, S. M. .. *Mycologia*, 1934, **26**, 294–99.

Explanation of Plate

1. Leaves of *Rhododendron nilagiricum* showing white growth of *E. butleri.*
2. Leaves of *Celtis tetrandra* with hymenia of *E. celtidis.*
3. Photograph of the herbarium material of *Gordonia obtusa* showing infection by *E. nilagiricum* on one of the leaves.
4. Basidiospore of *E. triseptatum* showing formation of secondary spores (× 1,000).

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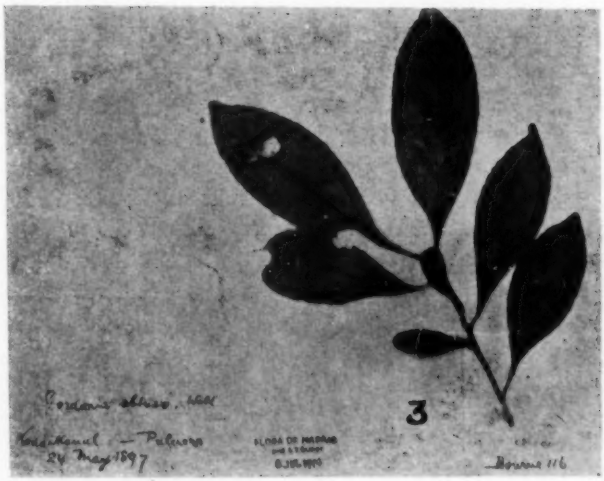
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STUDIES IN FISH LIVER OILS

Part I. Seasonal Variation in (*a*) Vitamin A Content, (*b*) Some of the Physical and Chemical Constants of Some Fish Liver Oils

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In Europe as well as in America, work on the seasonal variation in the yield and vitamin A content of the oil has been attempted by very many workers. Bills, *et al.* (1934), Haines and Drummond (1936), Evers, *et al.* (1936), Shortland (1938) and Molteno, C. J. and Rapson, W. S. (1939). In India work on the same line has been undertaken by Seshan (1940), Niyogi, *et al.* (1943 *a*) and Sarangdhar (1942).

It was observed in our laboratories that during the extraction of the oil from the livers of different species of fish that are available in Bombay coastal waters the colour of the oil varied from yellow to brownish red. On subjecting the various coloured liver oils for the assay of their vitamin A content using Lovibond tintometer great variations in the vitamin values for the same species were observed. It was also noticed that there was great variation in the colour as well as vitamin content from oils obtained from the livers of same fish collected and extracted at different periods of the year. It was observed that in the majority of cases the colorimetric assay gave more blue values for brown coloured oils than for pale yellow ones.

A careful study of the literature available revealed the fact that other than some stray references no systematic work has been done to correlate vitamin A value with different physical and chemical constants of the liver oils.

Embree (1939) has stated that cyclized vitamin A—the material formed by the treatment of vitamin A with hydrochloric acid in alcohol has been found to occur naturally in tuna and other fish liver oils. He further states that this cyclized vitamin A appears to have no growth-promoting power, but has considerable ultra-violet absorption at 328 μ and gives practically the same antimony trichloride reaction as vitamin A; he calls this 'Spurious vitamin A'.

Niyogi. Patwardhan and Acharya (1943 *b*), subjected the liver, oils from the two fish, *viz.*, mushi (*Scoliodon Sorrokowah*) and ghol (*Sciæna miles*)

to the tintometric and biological assay for vitamin A. They used the factor 4·2 (Bomskov, 1935) to convert the blue values (as read on the Lovibond tintometer) to International Units. The two methods gave values which agreed in the case of mushi liver oil, but in the case of ghol liver oil the biological value was however considerably lower than the tintometric result. From this observation they came to the conclusion that the vitamin A value obtained by tintometer is not always a true measure. They suggested that the ghol liver oil contained a chromogenic substance which did not promote growth; and this formed nearly a third of the chromogenic material responsible for the blue colour obtained with antimony trichloride.

As some of these liver oils are already in use as substitutes for cod-liver oil, it is of utmost importance to subject them for biological assay in order to see whether the whole of vitamin A estimated with antimony trichloride is available for growth or not. This study is presented in two parts. Part I deals with physical and chemical constants along with Vitamin A values determined by physico-chemical methods in liver oils and Part II deals with biological assay.

Six varieties of fish were taken in this investigation. Every month a sample of fish liver of each variety was collected, the oil extracted and used for the estimation of vitamin A by colorimetric, and physical and chemical constants by standard methods.

Experimental

Niyogi, *et al.* (*loc. cit.*) extracted the oil by the method of Carr and Price (1926) as modified by Coward, *et al.* (1931), for their investigation of the vitamin A content of fish liver oils. The finely minced liver was ground up with anhydrous sodium sulphate and extracted repeatedly with ether in the cold ; the ethereal extract was distilled in presence of nitrogen atmosphere and the oil was recovered. Traces of moisture were completely removed by drying the oil in vacuum over phosphorus pentoxide.

The method adopted for the extraction of the liver oil in this investigation was by steaming. Fresh livers were minced and mixed with twice their volume of water in a tinned copper vessel. The contents were cooked in a water-bath with constant stirring and keeping the temperature at about 80° C. The oil that oozed out was collected, filtered through a muslin cloth and transferred to Winchester bottles having taps at the bottom. The crude oil was allowed to settle for an hour to remove the water layer which was collected at the bottom of the Winchester bottles. To remove traces of moisture the oil was again transferred to the tinned copper vessel, gently

warmed in a water-bath and a small quantity of anhydrous sodium sulphate was added. The contents of the vessel were shaken vigorously and kept overnight in presence of nitrogen atmosphere. The clear oil was stored in coloured bottles or in iron drums in presence of an inert gas like nitrogen.

Estimation of Vitamin A

The method of Carr and Price (*loc. cit.*), as modified by Coward, *et al.* (*loc. cit.*), was adopted in this investigation for the estimation of vitamin A. A number of dilutions of oil was prepared in dry redistilled chloroform. The intensity of the colour produced when 0·2 ml. of each diluted solution was mixed with 2 ml. of antimony trichloride reagent in a cell was matched with Lovibond tinted glass the colour being matched within 30 seconds. Yellow and red glasses were frequently used to match the colour. The intensity of the blue colour on the Lovibond tintometer scale was so adjusted that it gave blue values between six and eight. Six or more readings were taken in each case and their mean found.

Since there was a change in the colour of the oil obtained from livers of the same species of fish collected at different seasons of the year, it was thought that there might be some change in some of the physical as well as chemical constants of these oils. The different constants of the liver oils mentioned above therefore were determined by standard methods month by month. The results are given in Table I below :

TABLE I

Showing some of the constants of six liver oils determined month by month

Sp. gr. at 30° C.	Acid value	Sap. value	Non-sap. matter	Acetyl value	Iodine value	Blue Units per gm. of the oil	Month of the year
OIL NO. 1. Local name of the fish : Khada mushi or Kondaicha Scientific name : *Calcarinus melanopterus*							
0·9200	0·622	166	8·452	26·50	128	12084	July (1944)
0·9206	0·610	166	8·634	26·60	127	13666	August
0·9200	0·620	169	8·906	27·30	126	17668	September
0·9196	0·610	171	8·984	27·60	126	21654	October
0·9198	0·620	173	8·063	28·20	125	30608	November
0·9201	0·613	166	8·238	26·60	135	10550	December
0·9200	0·686	165	8·240	26·66	133	10584	January (1945)
0·9201	0·623	165	7·986	26·30	137	9166	February
0·9209	0·706	165	7·989	26·32	136	9986	March
0·9190	0·726	166	8·322	26·40	130	11026	April
0·9198	0·646	166	8·323	26·52	129	11676	May
0·9198	0·658	167	8·587	26·64	129	12502	June
			Standard Deviation from Normal				
0·0135	0·0522	5·7	0·386	7·58	6·5		

TABLE I (*Continued*)

Sp. gr. at 30° C.	Acid value	Sap. value	Non-sap. matter	Acetyl value	Iodine value	Blue Units per gm. of the oil	Month of the year
OIL NO. 2. Local name of the fish : Waghbeer or Waghsheer Scientific name : *Gateocerdo figrinus*							
0·906	0·183	178	1·02	13·28	122	3087	July (1945)
0·913	0·192	182	1·05	13·46	121	3208	August
0·908	0·206	183	1·08	13·63	121	3386	September
0·912	0·186	184	1·12	14·86	119	3948	October
0·914	0·193	180	0·96	14·08	121	3269	November
0·912	0·188	177	0·82	13·09	123	2562	December
0·909	0·192	176	0·80	13·02	123	2286	January (1946)
0·910	0·196	176	0·86	13·42	126	2109	February
0·912	0·203	186	1·14	14·83	120	3846	March
0·912	0·198	183	1·04	14·46	121	3586	April
0·910	0·189	178	0·92	14·06	122	2987	May
0·912	0·193	179	0·92	13·82	123	2808	June
			Standard Deviation from Normal				
..	..	7·38	0·097	..	6·09		
OIL NO. 3. Local name of the fish : Kan mushi Scientific name : *Cestracion Blochii*							
0·908	0·186	176	1·178	41·88	132	8068	July (1945)
0·910	0·230	173	1·173	38·42	135	7606	August
0·916	0·220	174	1·178	39·08	134	7783	September
0·910	0·196	176	1·192	40·08	131	9342	October
0·920	0·206	178	1·192	44·86	122	12626	November
0·918	0·284	176	1·186	42·66	124	10862	December
0·910	0·326	175	1·183	40·87	136	9842	January (1946)
0·918	0·348	176	1·189	41·32	125	10686	February
0·920	0·326	178	1·190	41·86	124	11348	March
0·916	0·296	178	1·196	45·60	123	12746	April
0·920	0·280	178	1·206	45·63	123	12926	May
0·920	0·226	177	1·186	42·32	124	11366	June
			Standard Deviation from Normal				
..	0·184	11·45	0·169	8·48	..		
OIL NO. 4. Local name of the fish : Nali, Win, Sondel or Khandere Scientific name : *Pristis Perrotteti*							
0·908	1·862	186	3·68	13·86	114	4368	July (1945)
0·912	1·932	186	3·63	13·69	114	4308	August
0·915	1·676	188	3·75	13·78	115	4560	September
0·906	1·732	191	3·86	14·06	113	5086	October
0·912	1·286	190	3·84	13·16	115	4986	November
0·910	1·326	186	3·82	13·06	118	4063	December
0·915	1·084	186	3·84	12·96	118	4008	January (1946)
0·913	1·006	187	3·36	13·00	120	3986	February
0·908	1·086	188	3·80	13·68	118	4026	March
0·910	1·832	190	3·86	13·96	116	4580	April
0·913	1·892	190	3·78	14·11	115	4830	May
0·909	1·383	188	3·76	13·84	113	4683	June
			Standard Deviation from Normal				
..	0·357	..	0·276	..	18·72		

TABLE I—(*Continued*)

Sp. gr. at 30° C.	Acid value	Sap. value	Non-sap. matter	Acetyl value	Iodine value	Blue Units per gm. of the oil	Month of the year
OIL NO. 5. Local name of the fish : Wagli Scientific name : *Rhinoptera Javanica*							
0·912	0·680	183	2·48	22·98	117	1886	July (1945)
0·913	0·628	179	2·39	20·04	120	986	August
0·906	0·726	178	2·36	19·76	121	664	September
0·915	0·770	180	2·41	20·16	120	1096	October
0·912	0·655	181	2·50	22·86	118	1626	November
0·912	0·630	181	2·45	23·04	117	1728	December
0·915	0·710	183	2·45	22·06	118	1806	January (1946)
0·915	0·686	185	2·59	22·86	117	1988	February
0·914	0·686	186	2·72	23·42	116	2506	March
0·914	0·721	188	2·80	24·16	116	2626	April
3·913	0·658	186	2·68	22·06	116	2510	May
0·914	0·662	184	2·56	23·22	117	1968	June
			Standard Deviation from Normal				
..	0·658	..	0·2336	1·47	..		
OIL NO. 6. Local name of the fish : Surmai Scientific name : *Cybium Kuhlii*							
0·912	1·263	172	2·45	22·04	127	1347	July (1945)
0·909	0·982	174	2·48	22·18	126	1486	August
0·910	1·246	178	2·80	23·08	124	1792	September
0·908	0·962	178	3·10	23·86	123	1856	October
0·911	0·880	176	2·76	22·40	125	1646	November
0·912	0·906	174	2·54	21·86	126	1436	December
0·912	1·002	173	2·20	20·09	127	1208	January (1946)
0·910	1·182	172	2·12	19·78	128	1106	February
0·910	1·162	166	1·96	17·62	130	988	March
0·912	0·908	172	2·28	21·32	126	1289	April
0·908	1·020	174	2·66	22·48	125	1546	May
0·910	1·012	173	2·52	22·14	127	1354	June
			Standard Deviation from Normal				
..	0·140	..	0·314	2·16	8·28		

DISCUSSION

Slight variation in specific gravity and marked variations in acid value, saponification value, non-saponifiable matter and acetyl value can be observed from the six oils studied (refer Table I above) but in the case of the oils from the same fish studied month by month no such marked variations were observed. In the case of iodine value also it can be seen that there is variation not only in all the six varieties of the oils studied but also with individual oils studied month by month.

No relation was observed between the colour of the oils and their different physical and chemical constants; reference to this observation

will be made with quantitative data in Part II. Evers and Smith (1933) and Holmes, *et al.* (1941), state that vitamin A content of liver oils cannot be deduced with any degree of exactitude from mere knowledge of physical and chemical properties. Ganpule and Sarangdhar (1943) working on some of the chemical and physical constants of most of the commercially important sharks landed along the Bombay coast, have come to the conclusion that the physical and chemical constants by themselves furnish no clue whatsoever to the relative vitamin A potency of the respective oils.

From a critical study of the Table I above, in the majority of cases whether in the individual oils studied month by month or in all the six liver oils studied, the following may be observed: An increase in vitamin A content is followed by (*a*) an increase in saponification value, in non-saponifiable matter and in acetyl value, (*b*) no marked change in specific gravity and in acid values and (*c*) decrease in iodine number. The view that the increase in vitamin A is accompanied by decrease in iodine value has been recorded by Molteno and Raspon (1939) while working on seasonal variations in the vitamin A content of certain viscera of the Geelbek. They state that the iodine value varies inversely with the percentage of vitamin A in the oil.

The statements made above will be valid only when these results are found significant when they are treated statistically. Therefore the results were treated statistically and are given below in Table II below :

TABLE II

Constants	Number of the oil					
	1	2	3	4	5	6
	Standard Deviation from the Normal					
1 Specific gravity ..	0·01352	..	..	..	..	..
2 Acid value ..	0·05220	..	0·184	0·357	0·0658	0·140
3 Saponification value ..	5·71480	7·380	11·450	..	..	..
4 Non-saponifiable matter ..	0·38860	0·097	0·169	0·276	0·2336	0·314
5 Acetyl value ..	7·58800	..	8·480	..	1·4770	2·164
6 Iodine value ..	6·51000	6·090	..	18·720	..	8·280

From the above table it was deduced that some constants were found significant and others are not significant. These are shown below in Table III.

TABLE III

Constants	Number of the oil					
	1	2	3	4	5	6
	Statistical treatment of the above result					
1 Specific gravity ..	sig.	..	..	..	..	..
2 Acid value ..	sig.	..	not sig.	sig.	sig.	not sig.
3 Saponification value ..	sig.	sig.	sig.	..	..	..
4 Non-sap. matter ..	sig.	sig.	sig.	sig.	sig.	sig.
5 Acetyl value ..	sig.	..	sig.	..	sig.	sig.
6 Iodine value ..	sig.	sig.	..	sig.	..	sig.

N.B.—The formula used for treating the results statistically was that used by G. W. Snedecor in his book *Statistical Methods*. It was considered that the observed value was significant, if the observed value of t $\left(\text{i.e., } \bar{x}\Big/\frac{\sigma}{n}\right)$ is greater than the theoretical value of t (read on 't' (table) for $(n-1)$ degrees of freedom by 2% (*i.e.*, 2·178).

Besides an attempt has been made to correlate the vitamin A content with the different physical and chemical constants of the oils studied. The vitamin A content was found to be correlated with the logarithm of the (*a*) sap. value, (*b*) non-sap. matter, (*c*) acetyl value and (*d*) iodine value in the case of all the oils studied. Whereas in the case of Surmai relation could be traced between vitamin A content and logarithm of iodine value, saponification value and non-saponifiable matter only. No definite relation existed between vitamin A content and logarithm of specific gravity of oil except in the case of Nali. When these results (*i.e.*, vitamin A in Blue Units in thousands per gram against the logarithm of the different physical and chemical constants of all the liver oils studied) were plotted on a graph paper, a straight line passing through the maximum number of points was obtained. Graphs I to IV have been reproduced as specimen figures. The relationship may be expressed as follows:

$$\text{Vitamin A (B.U. in thousand per gm. of oil)} \quad .. \quad = K \log P + C.$$

Where K and C are constants and P is the physical or chemical constant of the oil.

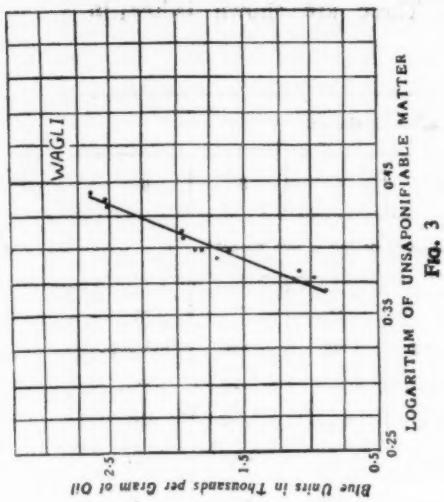

FIG. 3

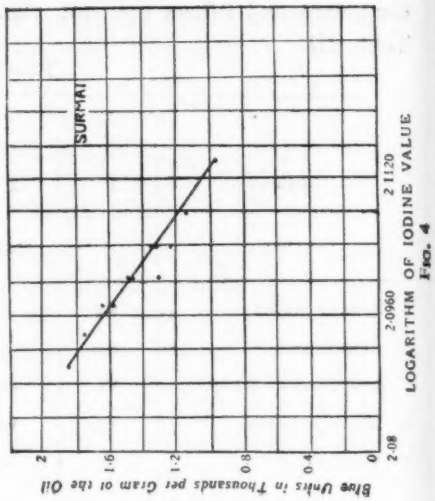

FIG. 4

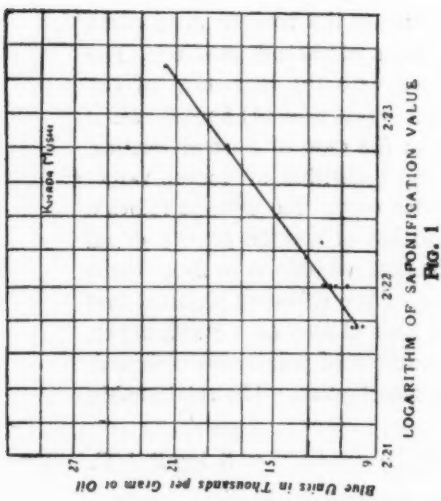

FIG. 1

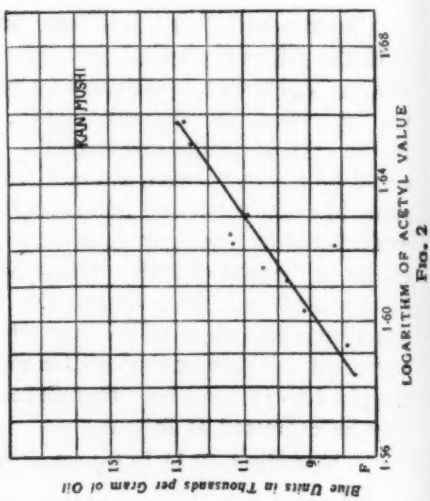

FIG. 2

For all the six liver oils studied the expressions obtained for different constants are given below :

Oil No. 1

Acid value	..	Vit. A (in B.U./gm. of oil)	$= 66 \cdot 6 \log P - 0 \cdot 79$
Sap. value	..	Vit. A („)	$= 160 \log P - 0 \cdot 44$
Non-sap. matter		Vit. A („)	$= 125 \log P - 0 \cdot 1$
Acetyl value	..	Vit. A („)	$= 600 \log P + 0 \cdot 6$
Iodine value	..	Vit. A („)	$= - 136 \cdot 36 \log P + 6 \cdot 13$

Oil No. 2

Acid value	..	Vit. A („)	$= - 4 \cdot 28 \log P + 1 \cdot 52$
Sap. value	..	Vit. A („)	$= 60 \cdot 97 \log P + 0 \cdot 77$
Iodine value	..	Vit. A („)	$= 29 \cdot 43 \log P + 0 \cdot 23$
Acetyl value	..	Vit. A („)	$= - 86 \cdot 2 \log P + 1 \cdot 94.$

Oil No. 3

Saponification value		Vit. A („)	$= 428 \cdot 6 \log P - 3 \cdot 05$
Non-sap. matter		Vit. A („)	$= 600 \log P - 2$
Acetyl value	..	Vit. A („)	$= 71 \cdot 4 \log P - 0 \cdot 99$
Iodine value	..	Vit. A („)	$= - 129 \log P + 8 \cdot 5$

Oil No. 4

Non-sap. matter		Vit. A („)	$= 26 \cdot 09 \log P - 0 \cdot 96$
Specific gravity	..	Vit. A („)	$= 235 \cdot 3 \log P - 0 \cdot 54$
Acetyl value	..	Vit. A („)	$= 15 \cdot 4 \log P + 0 \cdot 36$

Oil No. 5

Saponification value		Vit. A („)	$= 83 \cdot 3 \log P - 0 \cdot 49$
Non-sap. matter		Vit. A („)	$= 21 \cdot 43 \log P - 2 \cdot 07$
Acetyl value	..	Vit. A („)	$= 10 \log P + 0 \cdot 6$
Iodine value	..	Vit. A („)	$= - 105 \cdot 26 \log P + 2 \cdot 55$

Oil No. 6

Saponification value		Vit. A („)	$= 35 \cdot 3 \log P + 0 \cdot 41$
Non-sap. matter		Vit. A („)	$= 4 \cdot 7 \log P + 1 \cdot 05$
Iodine value	..	Vit. A („)	$= - 37 \cdot 5 \log P + 2 \cdot 23$

Summary and Conclusion

With a view to finding out whether there exists any relationship among the colour of the oil and its different physical and chemical constants and vitamin A content, liver oils were obtained from six varieties of fish available in the Bombay coastal waters. These fish yielded a large amount of oil

with high vitamin A content. The oil was extracted and analysed month by month and the different physical and chemical constants of the oil along with vitamin A content were investigated. It is found from the table that (1) there is no relation between the colour of the oil and its different constants. (2) An increase in vitamin A content is followed by (*a*) an increase in the saponification value, in non-saponifiable matter and in acetyl value, (*b*) no marked change in specific gravity and in acid values and (*c*) decrease in iodine number. When these results were treated statistically, only the alterations in acid values of Waghbeer and Surmai were found statistically not significant.

An attempt to obtain an expression to correlate the vitamin A content in B.U. and the logarithm of the physical and chemical constants of the liver oils studied has been made. The expression obtained being:

Vitamin A (in Blue Units per gm. of oil) = K log P + C, where K and C are constants and P is the physical or the chemical constant. Values for K and C are different for the different oils studied.

References

Bills, C. E., Imboden, Miriam, and Wallenmeyer, J. C. — *J. Biol. Chem.*, 1934, **10**, 105, Proc. 28.

Bomskov, C. .. *Methodik der vitaminforschumg*, Georg. Thiems, Leipzig, 1935, 63.

Carr, F. H., and Price, C. F. .. *Biochem. Jour.*, 1926, **20**, 497.

Coward, K. H. .. *Ibid.*, 1931, **25**, 1102.

Embree, N. D. .. *J. Biol. Chem.*, 1939, **128**, 187.

Evers, N., Jones, A. G., and Smith, W. — *Analyst*, 1936, **7**, 61.

Evers, N. and Smith, W. .. *Quart. J. Pharm.*, 1933, **6**, 329.

Ganpule, N. V., and Sarangdhar, P. N. — *Jour. Ind. Chem. Soc., Ind. and News Edn.*, 1943, **6**, 161.

Haines, R. T. M., and Drummond, J. C. — *Analyst*, 1936, **2**, 61.

Holmes, A. D., and Tripp, F. .. *Ind. Eng. Chem.*, Ind. Edn., 1941, **33**, 944.

Molteno, C. J., and Rapson, W. S. — *Biochem. J.*, 1939, **33**, 1390.

Niyogi, S. P., Patwardhan, V. N., and Acharya, B. N. — *Ind. Jour. Med. Res.*, 1943, **31**, 21.

———— .. *Ibid.*, 1943, **31**, 15.

Sarangdhar, P. N. .. *Ibid.*, 1942, **30**, 553.

Seshan .. *Ibid.*, 1940, **27**, 711.

Shortland, F. B. .. *Biochem. Jour.*, 1938, **32**, 488.

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STUDIES IN THE ANATOMY OF SUGARCANE STALK*

Part II. Milling Characteristics of Varieties

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I. Introduction

In sugarcane, all the matter insoluble in water is known as fibre, the quantity of which is distinctly a varietal character affected, to some extent, by the age and conditions of growth. That the efficiency of milling was determined by this quantity was well known long time back, and as a general rule, it was stated that the higher the fibre content of a cane was, the greater was the difficulty in milling it.

It was only recently that the effect of the quality of fibre was noted. Dymond (1942) found that although Co 290 contained less of fibre than Co 281, the former was markedly inferior to the latter, because the fibre of Co 290 retained 24–25% more of juice per unit weight of fibre than that of Co 281. Puertas (1943) also noted that juice was more easily expressible from the fibre of some varieties than from that of others. Lower extraction necessitates addition of greater amount of water of imbibition, to evaporate which larger quantity of fuel is required, with the result that the cost of production is increased, unless the variety yields sufficient bagasse for the purpose. Besides, the quality of fibre affects the economy of factory working also directly in that the bagasse of some varieties is good as fuel, because it burns well and is sufficient for the purpose. Camden Smith as reported by Hedlay (1936) found that bagasse of varieties like Co 301, P.O.J. 2714 and P.O.J. 2725 did not burn well. Dymond (*loc. cit.*) noted that the bagasse of Co 290 was poorer than that of Co 281, so far as its steam raising quality was concerned.

It thus becomes essential to determine both quantity and quality of fibre so that these characteristics receive due consideration in the score-card for ultimate selection of seedlings. As would appear from what has been stated above, the problem concerns largely with the study of structural variations in different varieties and in order, therefore, to understand how far

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the anatomical makeup of a cane stalk would be an index of its milling propensities, varieties dominant at one time or other in the province, *viz.*, Co 210, Co 213, Co 299, Co 313 were selected for this study. Co 419 and Rheora, the latter being one of the main varieties before the introduction of Coimbatore canes, were also included because considerable difficulty in obtaining maximum extraction from them was generally reported from different factories. The bagasse of these varieties was also found to contain uneconomical quantities of sugar. These six varieties together covered fairly wide range of milling characteristics.

The present contribution deals only with the quantity of fibre in relation to major anatomical features. Besides, an attempt has been made to set out the problem in its various details with a view to focus pointed attention of workers in the line.

II. Material and Methods

Transverse sections taken from middle internodes of normal healthy looking stalks of each variety were stained in 1% solution of safranin in 50% alcohol and mounted in Canada balsam after the usual process of dehydration. In the preliminary stage the following characters were taken into consideration.

1. Vascular Bundles—Average number and size in (*a*) peripheral and (*b*) central regions.

2. Thickness of cell walls in (*a*) Parenchymatous matrix at 0·6 mm. and 1·2 mm. from epidermis and in centre and (*b*) Sclerenchymatous vascular sheaths in peripheral and central regions.

The number and size of vascular bundles were noted in 48 unit areas (the field of microscope being taken as a unit) in each region. In order that the unit areas were comparable to one another in different varieties, care was taken to keep the edge of a microscope-field always touching the epidermis. The size of vascular bundles was measured in terms of radial and tangential axes, the product of which gave a highly reliable idea of its area in cross-section, as reported elsewhere (Khanna and Sharma, 1947). The thickness of cell walls was measured for two adjacent walls in both the tissues, averages being taken from 16 readings for each tissue at each depth.

III. Results of Investigations

The six varieties selected for the study could be placed (Table I) in three distinct groups, *viz.*, (i) the high-fibre group having Co 299 as an example (ii) the medium-fibre group to which Co 210, Co 213 belonged and (iii) the low-fibre group which included Co 313, Co 419 and Rheora.

TABLE I

Showing average fibre % cane of the six sugarcane varieties in different months

Months	Varieties						Mean
	Co 210	Co 213	Co 299	Co 313	Co 419	Rheora	
November ..	14·84	13·95	16·24	12·96	11·60	11·00	13·43
December ..	15·33	14·40	16·66	13·38	11·99	11·38	13·86
January ..	15·78	14·82	17·40	13·90	12·53	11·89	14·38
February ..	16·57	15·58	18·04	14·60	13·13	12·49	15·07
March ..	17·50	16·19	18·71	15·40	13·83	13·10	15·79
April ..	18·34	16·98	19·70	16·06	14·47	13·93	16·58
Average ..	16·39	15·32	17·79	14·38	12·92	12·30	14·85

S.E. per plot (month) = ±0·6571 or 4·42% of the mean.
S.E. (V) = ±0·06707.
S·E. (P×V) = ±0·1643.
C.D. at 5% = 0·1858.
C.D. at 1% = 0·2442.
At 5% Co 299, Co 210, Co 213, Co. 313, Co 419, Rheora
At 1% Co 299, Co 210, Co 213, Co 313, Co 419, Rheora.
Conclusion : The varietal differences are highly significant.

(*a*) *Vascular bundles.*—The detailed examination of slides in respect of the two characters studied brought out (Table II) that the differences among the varieties of the first and second groups and Co 313 of the low-fibre group (Plate II, Figs. 1–4) were not significant so far as the numbe· of vascular bundles per unit area in peripheral region was concerned, while Co 419 and Rheora although falling in the same group occupied the two extremes (Plate II, Figs. 5 and 6) and were significantly different from all the four varieties, *viz.*, Co 210, Co 213, Co 299 and Co 313. As regards the size, Co 419 and Rheora had respectively the largest and smallest vascular bundles. The differences in the size were significant at 5% level in all varieties except Co 299 and Co 213. When the two characters of vascular bundles were considered together as reflected by the total area occupied by them in unit area of parenchymatous ground tissue, Co 419 and Co 210 had approximately the same area while Rheora was nearer Co 213 and Co 299 than Co 419. Co 313 occupied an intermediate position.

In the central region also, Co 419 and Rheora occupied the two extremes in respect of both the characters discussed above, and were significantly different when they headed the list in a particular character. Rheora and Co 419 had respectively the largest and the smallest number of vascular

TABLE II

Showing anatomical characters of six varieties of sugarcane

Characters	Co 210	Co 213	Co 299	Co 313	Co 419	Rheora	General mean	C.D. at 5%
A. *Vascular bundles*								
I. *In peripheral region* :								
(*a*) No. per unit area								
Actual ..	9·17	8·64	9·00	8·96	7·71	14·98	9·74	0·58
% of total ..	15·7	14·8	15·4	15·3	13·2	15·6	..	..
(*b*) Size—								
Actual ..	404·4	316·4	324·8	367·8	485·0	188·5	347·8	32·7
% of total ..	19·4	15·2	15·6	17·6	23·3	9·1	..	..
(*c*) Total area occupied by vascular bundles in unit area								
Actual ..	3708	2734	2932	3295	3739	2824	..	..
% of total ..	19·3	14·2	15·2	17·1	19·5	14·7	..	..
II. *In Central region*:								
(*a*) No. per unit area								
Actual ..	3·44	3·73	3·02	3·14	2·75	4·48	3·43	0·32
% of total ..	16·7	18·1	14·7	15·3	13·4	21·8	..	..
(*b*) Size—								
Actual ..	359·6	306·0	346·3	362·6	486·5	281·4	356·5	34·4
% of total ..	16·8	14·3	16·2	16·9	22·7	13·1	..	..
(*c*) Total area occupied by vascular bundles in unit area								
Actual ..	1237	1141	1046	1139	1328	1261	..	..
% of total ..	17·3	16·0	14·6	15·9	18·6	17·6	..	..
B. *Thickness of cell walls*								
I. *Parenchyma* :								
(*a*) At 0·6 m.m.—								
Actual ..	23·12	23·31	28·31	11·56	14·62	12·12	18·84	3·25
% of total ..	20·4	20·6	25·1	10·2	12·9	10·7	..	..
(*b*) At 1·2 m.m.—								
Actual ..	23·69	21·81	24·25	11·56	12·81	12·50	17·77	2·19
% of total ..	22·2	20·5	22·8	10·8	12·0	11·7	..	..
(*c*) In centre—								
Actual ..	13·94	13·56	17·19	13·44	11·31	12·62	13·68	1·64
% of total ..	17·0	16·5	20·9	16·4	13·8	15·4	..	..
II. *Sclerenchyma* :								
(*a*) In peripheral region:								
Actual ..	54·0	39·31	64·56	29·94	23·62	21·75	38·94	4·77
% of total ..	21·2	16·9	27·7	12·8	10·1	9·3	..	..
(*b*) In centre—								
Actual ..	18·37	17·50	25·25	21·19	12·25	13·81	18·06	3·11
% of total ..	17·0	16·2	23·3	19·6	11·3	12·7	..	

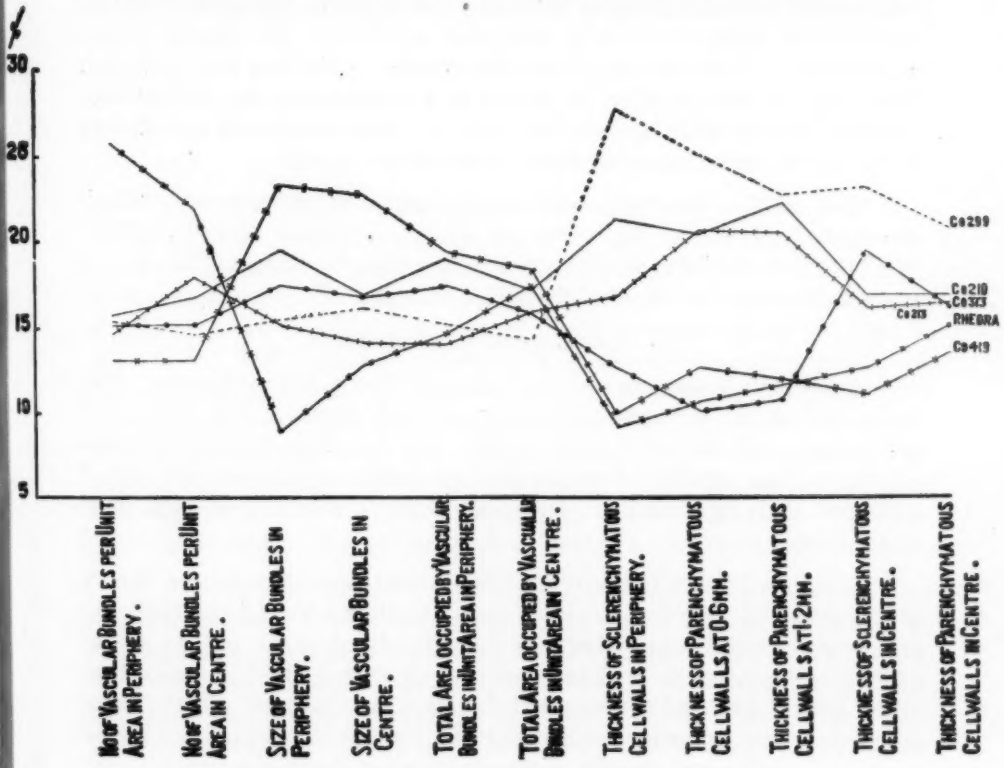
Note.—1. The field of the microscope measuring 1·7 mm. in diameter was taken as unit area of parenchymatous matrix in which the number of vascular bundles were counted.

2. All measurements were expressed in divisions of eye-piece micrometer each of which was equal to $14 \cdot 4^2$ sq. μ and 0·21 μ respectively for the size of vascular bundles and the thickness of cell walls.

bundles per unit area, but their position was reversed when the size was considered. Of the remaining four varieties, Co 210, Co 299 and Co 313

formed a compact group while Co 213 served as a link between them on one hand and Rheora on the other, so far as the size was concerned, while in respect of the number per unit area, there was no clear grouping amongst the six varieties studied. On the basis of total vascular area also, there were no well-demarcated groups except that Co 299 and Co 419 were distinctly set apart at the two ends. The varieties of the two groups having medium and low fibre were, therefore, found scattered so far as the characters of vascular bundles were concerned.

(*b*) *Thickness of cell-walls.*—These varieties, however, segregated into three groups, more or less compact in the case of the low-fibred ones (Fig. 1)


30
28
20
15
10
5
Co 299
Co 213
Co 313
Co 419
RHEORA
No. of Vascular Bundles per Unit Area in Periphery.
No. of Vascular Bundles per Unit Area in Centre.
Size of Vascular Bundles in Periphery.
Size of Vascular Bundles in Centre.
Total Area occupied by Vascular Bundles in Unit Area in Periphery.
Total Area occupied by Vascular Bundles in Unit Area in Centre.
Thickness of Sclerenchymatous Cell-walls in Periphery.
Thickness of Parenchymatous Cell-walls at 0·6mm.
Thickness of Parenchymatous Cell-walls at 1·2mm.
Thickness of Sclerenchymatous Cell-walls in Centre.
Thickness of Parenchymatous Cell-walls in Centre.

TEXT-FIG. 1. Anatomical Characters of Sugarcane Varieties.

when the thickness of cell walls was taken into consideration. Co 299 representing the high-fibre group was a class by itself, being quite distinct in that it had very high lignification all over the entire cross-section (Plate II,

Fig. 1). Co 210 and Co 213 together formed a group as they were not significantly different from each other except in the thickness of cell walls in vascular sheaths in the peripheral region. Taking the total lignification of both the tissues—the parenchyma and sclerenchyma into consideration, Co 210 came next to Co 299 and Co 213 after it (Table II).

In the low-fibred group, Co 419 and Rheora were not significantly different from each other and showed on the whole, the poorest lignification of the tissues. Co 313 was found to be erratic in its behaviour. Whereas the variety was poorest in the lignification of the parenchymatous matrix of the rind, it was proportionately high so far as the tissues of the core were concerned. The sclerenchyma in centre was as highly lignified as that of Co 210 and significantly more than that of Co 213. In respect of the parenchymatous ground tissue it was significantly higher than that in Co 419. The variety might, therefore, be treated as a link between the medium-fibre group consisting of Co 210 and Co 213 on one hand and Co 419 and Rheora group on the other, but much closer to the latter.

The varieties, therefore, could be arranged in ascending order of lignification of tissues on the whole as follows: (Rheora–Co 419)–Co 313–(Co 213–Co 210) Co 299 which agreed closely with the sequence of varieties when arranged on the basis of their fibre contents.

IV. Enunciation of the Problem

The utility of sugarcane in the factory is determined by the ease, with which it yields its juice and the facility with which the latter in its turn, gives up the required product, namely sugar. The two-distinct phases of processing the raw material into the finished article, are respectively called extraction and condensation. The present paper naturally confines itself to the former only.

Extraction of juice is a very complex process depending upon a variety of factors which may be grouped together as (i) mechanical pertaining to (*a*) the opening between rollers, (*b*) their peripheral speed and roughness of their surfaces, (*c*) the hydraulic pressure on them and (*d*) the curvature of the trash turner, and (ii) botanical dealing with sugarcane. Of the latter group which may be further subdivided into (*a*) morphological and (*b*) anatomical—the quality and quantity of fibre in cane are the most important ones to be taken into consideration.

A stalk of sugarcane from a miller's point of view may be considered as a composite recepticle made of innumerable small containers—the cells—more or less cylindrical in shape, each of which must be ruptured to yield

its contents, either by mechanical pressure or by heat. The peripheral layers of cells of the stalk constitute a jacket—the rind—which gives the necessary protection to the inner cells and requisite strength to the whole structure. The hard covering may be deemed to have protruded inwards so as to form partitions—the nodes—to give further strength to the stalk. It is, therefore, essential to know how much and what type of material has been used to form the walls of the containers, the cells, the strengthening jacket, the rind, and the transverse partitions—the nodes.

This together with certain contents of the cells which are insoluble in water, constitutes fibre in sugarcane of which in its Lousiana variety the main components according to Brownie and Blouin as cited by Deerr (1921) were:

	In rind %	In core %
Cellulose	.. 51·0	49·0
Pentosans	.. 26·9	32·0
Legnin	.. 17·2	15·0

Within varietal limits the quantity of fibre is known to vary according to the age and environment. But regarding quality nothing definite can be stated at present although on theoretical considerations it is not likely to vary to any appreciable extent. Similarly whether the exact composition of fibre in a mature stalk is susceptible to the influences of environment is not known. The behaviour of cane stalk in various operations during the process of extraction and the utility of the resultant bagasse as fuel in relation to its morphological and anatomical characters, need much systematic work in this country before operating peculiarities of a cane variety can be forecast in its infancy. Below are given some of the difficulties often met with, depending upon how far the machinery is easily adjustable to suit the new requirements, when a change-over from one variety to another takes place.

Preparation of cane.—During the preparatory stage the cane stalk is cut up into very small pieces before passing through the rollers. Montgomery (1936) reported the experience of Alexander Smith who found that a closer setting of knives was necessary for short stalked variety D 1135 than that required for Yellow Calendonia. At Chanpatia and Motihari knives had to be adjusted nearer together while preparing Co 395 and B.O. 3. As a result of this preparation, certain varieties, for instance, B.O. 3 were reduced to a finely divided pulp, while in others like B.O. 9 the texture of the blanket was coarse. The effects of this differential disintegration would be discussed in their appropriate places.

Feeding.—The rate of feeding the rollers is mainly determined by the grip that the roller is able to maintain on the blanket of the prepared cane. If the cane is very finely divided, the rollers are unable to take a feed with the result that chocking takes place because the rollers slip over the blanket without pushing it forward. This feeding difficulty is due to low fibre content of the cane as was found to be the case with Co 419 or to its very fine division in the preparatory process, *e.g.*, in B.O. 3. Both the causes weaken the cohesion of the resultant blanket. In B.O. 9, no feeding difficulty was experienced because of the coarse texture of the blanket which provided a good grip. In addition to its effect on dry crushing, the degree of fineness of preparation determines the quantity of water of imbibition that can be safely used. The finer the disintegration, the less the quantity of water that can be left in to leach out remaining juice from the pulp because the quantity of water being the same, a finely textured blanket provides a much poorer grip to the rollers than a coarse-textured one.

Extraction (proper) is the operation when the prepared cane pulp is pressed in between the rollers to separate the liquid and solid components of the blanket. That other factors namely the opening between the rollers, their peripheral speed and hydraulic pressure on them, being the same, the volume of fibre in the blanket determines the extraction, is well known; so also its effect on the movement of bagasse on the trash turner. The quality of fibre comes into the picture in certain varieties in which, for instance, Co 513 the fibre tends to fur the rollers resulting in the lifting of scraper plates of the back and top rollers. Also, in the absence of adequate cohesion in blanket, cush-cush falls in juice thus overloading the juice strainers. Finally with the same pressure bagasse of certain varieties retains greater quantity of moisture than that of others.

Addition of water of imbibition.—The economic quantity of water of imbibition is determined by (i) the juice holding capacity of the fibre, (ii) the texture of the blanket and (iii) the availability of fuel in the shape of bagasse of which the first two factors are mainly a function of the quality of fibre while the last named that of both its quality and quantity. Provided the blanket is cohesive enough to flow, varieties rich in fibre of good thermal value, admit of greater quantity of water of imbibition resulting in more thorough leaching of bagasse and therefore higher milling efficiency which in the ultimate analysis may be defined as

$$\frac{100 \times \text{sugar extracted}}{\text{total sugar per cent. in cane.}}$$

Bagasse as Fuel.—Bagasse in order to be useful as fuel in the boiler room should not be so fine as to be wafted away through the chimney nor

should it be so coarse as to contain flakes which would retard combustion. It should contain adequate quantity of moisture which according to Alexander Smith as reported by Hedlay (1936) was found to be 40%. These desirable qualities of bagasse together with the thermal value and resultant absence of clinkers in furnace are directly the attributes of the quality of fibre. That quantity would determine whether bagasse is sufficient or not, is too obvious to be mentioned.

V. Discussion of Data

From the observations recorded earlier it would appear that the number of vascular bundles per unit area and their size were not factors of sufficient importance to explain the differential behaviour of the six varieties when they were milled. The total area under them per unit area in the two regions in different varieties also was in no way helpful in understanding their divergent milling properties. As these varieties segregated into three groups, when the lignification of cell-walls of two tissues namely parenchyma and sclerenchyma in different regions was taken into consideration, thickness of cell walls could, therefore, be taken as a sufficiently good index of fibre in cane. Buzacott (1940) and Rao (1941) also found a positive correlationship between the fibre content and rind hardness which in the ultimate analysis was found to be the function of the lignification of cells forming the vascular sheaths and of parenchymatous matrix as also of the area under vascular bundles (Khanna and Panje, 1939).

Turning to the milling characteristics of these varieties, Co 210 and Co 213 were found to posses the same operating peculiarities which appeared to be quite natural in view of their fibre content. The change-over from one variety to the other did not involve any adjustment of mill settings because both the canes were very similar to each other in their anatomical characters.

With the introduction of Co 299, the mill settings had to be adjusted to suit the greater volume of fibre in cane. Once a satisfactory adjustment was arrived at, no further trouble was experienced and the resultant bagasse was more than sufficient. All these features to a large extent could be foreseen from the much greater lignification of tissues in this variety than in Co 210 and Co 213.

In the case of Co 313 for which a large number of mill tests have been made during past nine years (1939–40 to 1947–48), only in a few cases a certain amount of difficulty, largely due to low fibre content, was met with. Its bagasse was reported to be insufficient in six cases: it was light but burnt well. Addition of comparatively large quantity of water of imbibition required for economic recovery, sometimes resulted in slipping or choking

of rollers. The position of this variety in respect of the lignification of tissues explained all these peculiarities except the fineness of bagasse and necessity of large quantity of water of imbibition which were the attributes of the quality of fibre.

Milling of Co 419 was found to bristle with difficulties at every stage. It gave trouble throughout in almost all the milling tests. The cane broke under the impact of knives and was inclined to choke splitters; also the feed was very irregular due to insufficient cohesion of the blanket which therefore, often choked the rollers. Some factories reported uneconomically high polarity of bagasse while others found it simply impossible to crush it alone with the then settings which in almost all cases were adjusted for medium-fibred canes like Co 210 and Co 213.

It would not be out of place to discuss exactly why a low-fibred variety could not be milled efficiently with mechinery set for medium-fibre canes. As the blanket of prepared canes approaches the top and back rollers, it is broken up into its solid and liquid components, namely fibre and juice the former of which together with a small quantity of the latter is thrown out while the greater part of the latter retained. The mass thus ejected consists of a solid residue, the fibre and a fluid residue the juice, and is equal to the " escribed " volume which is obtained by multiplying the width of the opening between the rollers, by their length and the surface speed (Deerr, 1921). Now " escribed " volume remaining constant, the quantity of juice allowed to pass through the rollers will be greater if the quantity of the solid residue is reduced. Again in order to obtain maximum extraction, it is essential that compressed bagasse between the coacting rollers forms a juice-tight seal so that the extracted juice is not reabsorbed by the bagasse which has passed the maximum pressure between the rollers. Consequently reduction in the amount of fibre in the emergent material would allow more of juice to be reabsorbed and this together with inadequate expulsion of juice would explain higher polarity of the bagasse.

Moreover, as stated before the amount of fibre determines the pressure on trash tuner and thereby the speed of the blanket. Mill setting remaining the same, a decrease in solid residue will therefore, reduce the pressure on the trash turner and a blanket of finely prepared bagasse will not be cohesive enough to maintain a regular feed for the top and back rollers.

In view of what has been stated above, low fibre content explained all the difficulties experienced while milling Co 419. Rheora in which total lignification was estimated to be more or less equal to that of Co 419 had exactly the same operating peculiarities. According to Sinclair (1941) it

was reduced to such a fine state in the final bagasse that much of it was blown away through furnace without proper combustion; also the polarity of final bagasse was higher than in Coimbatore varieties.

Summary

1. The present contribution deals with milling characteristics of six sugarcane varieties in relation to their anatomical make-up.

2. A short review of literature is given. Whereas the influence of fibre content on efficient milling was known long time back, that of the nature of fibre has been realised only recently. The problem in its various details is enunciated.

3. Six varieties, *viz.*, Co 299 with high fibre, Co 210 and Co 213 having medium fibre and Co 313, Co 419 and Rheora with low fibre were studied for the following characters.

A. The number of vascular bundles per unit area and their average size in a unit area in (i) the peripheral and (ii) central regions.

B. The thickness of cell-walls of (i) parenchymatous tissue at depths of 0·6 mm. and 1.2 mm. from the epidermis and in central region and (ii) of sclerenchymatous tissue in the periphery and centre.

4. The number of vascular bundles per unit area and their size, and total area under them as such, did not seem to explain the differential behaviour of these varieties when milled.

5. Co 299 was found to have the greatest amount of lignification of cell-walls over the entire cross-section; as also the fibre. Co 210 and Co 213 came next to it from both points of view. Co 313 was intermediate between the medium-fibred group and Co 419 and Rheora which showed poorest lignification and lowest fibre.

6. The magnitude of lignification in both the tissues in different regions was found to be a reliable index of fibre in cane.

7. The operating peculiarities of the six varieties were explained on the basis of fibre content of the cane and therefore on the magnitude of lignification of both the tissues. Co 210 and Co 213 could be milled efficiently with the same settings. Co 299 required regulation of speed because of higher fibre content. In the case of Co 313, some trouble was experienced because of rather low fibre percentage. Milling of Co 419 and Rheora was found to bristle with difficulties. Their bagasse was not sufficient; there were frequent choking at splitters or rollers; and the polarity of the

bagasse was uneconomically high, all of which could be explained by poor lignification of tissues.

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REFERENCES

1. Buzacott, J. H. .. "The Relationship between Hardness of Sugarcane and Varietal Resistance to Beetle Borer (*Rhabdocnemis obscure* Boisd)," *Tech. Commun. Bur. Sug. Expt. Sta.*, Queensland, 1940, **8**, 127–52.
2. Deerr, Noel .. *Cane Sugar*, Norman Rodger, London, 1921.
3. Dymond, G. C. .. "Varietal milling results in Natal," *Proc. 16th Cong. S. African Sugar Tech. Assoc.*, 1942, 37–42.
4. Hedlay, E. P. .. "Some physical properties of sugarcane," *Proc. 10th Cong. S. African Sugar Tech. Assoc.*, 1934, 39–47.
5. Khanna, K. L., and Panje, R. R. "Studies in the Rind Hardness of Sugarcane. I. Anatomy of the Stalk and Rind Hardness," *Ind. Jour. Agric. Sci.*, 1939, **9**, 1–14.
6. ——— and Sharma, S. L. "Studies in the anatomy of sugarcane stalk.—Milling characteristics of varieties," *Proc. Ind. Sci. Cong.*, 1940, 46–47.
7. ——————— .. "Studies in the anatomy of sugarcane stalk. I. Chewing canes," *Proc. Ind. Acad. Sci.*, 1947, **24**, 13–31.
8. Montogomery, J. W. .. "Milling and boiling characteristics of new varieties," *Proc. 15th Hawai Sug. Tech. Associ.*, 1936, 189–97.
9. Puertas, R. P. .. "A study of cane fibre, its composition and its effects on milling efficiency," *Proc. 17th Meeting Associ. Sugar Tech.*, Cuba, 1943, 95–110.
10. Thuljaram Rao, J. .. "Rind hardness as a possible factor in resistance of Sugarcane Varieties to the stem borer," *Curr. Sci.*, 1941, **10**, 365–66.

EXPLANATION OF PLATE II

T.S. of peripheral region of stems of six sugarcane varieties.—Fig. 1, Co 299 ; Fig. 2, Co 210; Fig. 3, Co 213 ; Fig. 4, Co 313 ; Fig. 5, Co 419 ; and Fig. 6, Rheora. Figs. 1 and 2 show high lignification of cell walls, more so in the former where the lumen of cell in vascular sheaths is hardly visible ; the vascular bundles are well defined. Fig. 3, cell walls are not so thick as in the first two : vascular bundles imperceptibly merge into ground tissue. Fig. 4, lignification is not so great as in the first three. The lumen of cells is distinctly present ; vascular bundles in all the four are more or less equal in size. Figs. 5 and 6, similar to Fig. 4. Vascular bundles are largest but smallest in number in Fig. 5, and the reverse in Fig. 6 (×80).

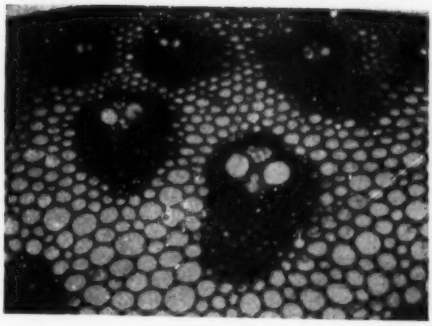

FIG. 1

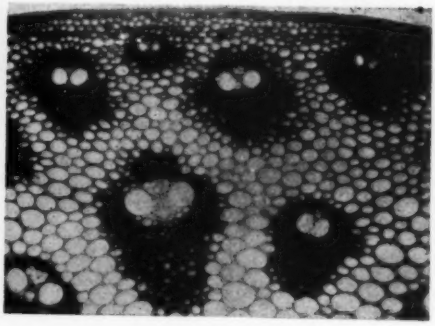

FIG. 2

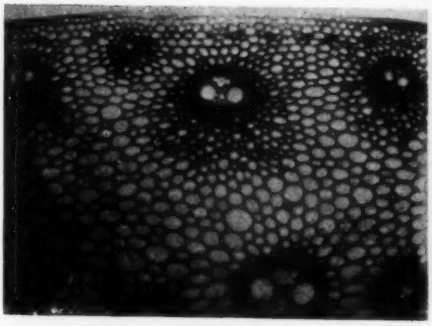

FIG. 3

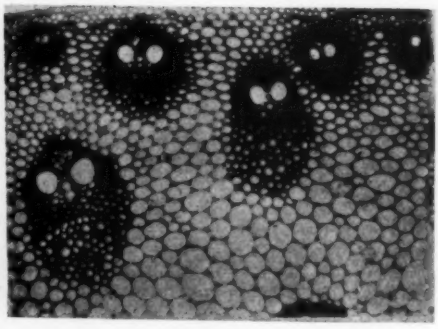

FIG. 4

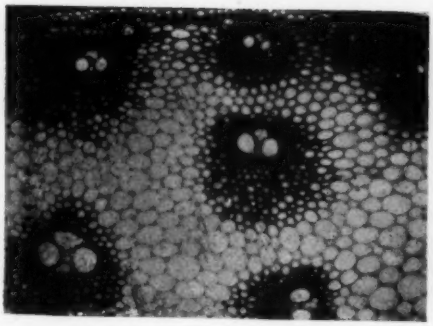

FIG. 5

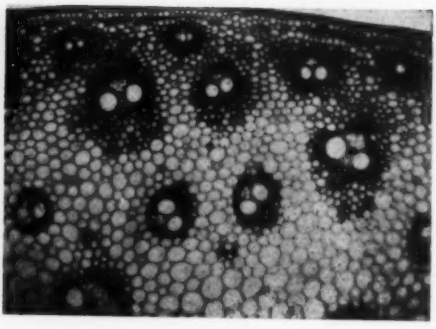

FIG. 6

STUDIES IN THE ANATOMY OF SUGARCANE STALK

III. Fission and Pseudo-Fission

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I. Introduction

While harvesting B. O. 17 (a variety bred at the Station) the authors came across an abnormal case of bifurcation of stalk in which the two arms, after having grown independently for some distance, appeared to have coalesced to form a single stalk again.

Normally once a fission had taken place, the two resulting stems maintained their separate existence to the end.

With a view to study this abnormal case, search for normally bifurcated stalks yielded one stalk of Co 213 and two of *Saraitha*, a variety of indigenous canes of the province, belonging to the group of the same name.

All the four stalks were described in detail both from morphological and anatomical points of view, and an effort was made to explain the phenomenon.

II. Material and Methods

The four stalks were numbered as follows:

B.O. 17	.. I
Co 213	.. II
Saraitha	.. III and IV (Plate III).

The internodes were counted upwards and downwards respectively as + 1, + 2 and so on, and − 1, − 2 from the node above which the two arms existed as independant entities and which was, therefore, designated as Node 0. The nodes were similarly numbered as + i, + ii and so on upwards and − i, − ii.... downwards from Node 0. The two halves or arms of a stalk to the left and right of the median diameter* were respectively known as A and B. In the following account the number of a stalk is

* A stalk of sugarcane has two principal diameters, namely, (i) the median passing from front to back through the bud and (ii) the lateral from side to side at right angles to the median. If median diameter is greater than the lateral, the cross-section is said to be oval; if reverse is the case, it is known as flattened.

followed by that of an internode or a node; thus I/ + 2B would mean second internode of the right arm above Node 0 of stalk I. After morphological description of the stalks, their hand sections were taken from different internodes and at different levels of the same internode when necessary, were stained with 1% solution of safranin in 50% of alcohol, and mounted permanently.

III. Morphology

(*a*) *Normal fission.*—The two stalks of *Saraitha* were similar in that they did not bear within three or four internodes any outward indication of the agency causing the fission, and that the resultant arms of the fork were more or less equal in length and girth at least for a couple of internodes above the node 0 where actual rupture had taken place. In both the cases the process of splitting was slow and spread over 2–3 internodes below the node 0.

III/ – 4 had no signs of the oncoming split, as it was more or less circular in cross-section having only one bud groove and a cavity in the centre. At III/ – iii there appeared three buds of which the central one was much smaller than those, situated laterally and more or less equal in size. The left bud had a distinct furrow above it while there was no indication of a bud groove on the right, the central bud being too small to have any groove. III/ – 3 was somewhat flattened in cross-section, but the cavity inside maintained its central position. At III/ – ii there were two buds more or less equal in size but only the right one had a bud groove. III/ – 2 above it showed further flattening and the central cavity was pushed to the right and a small cavity was found to develop slowly in the left portion. Still there was no indication of any fissure on the surface. On III/ – i however, the buds were situated on either side of a cleft, and had their own grooves. III/ – 1 showed well developed fissures at both the ends of the median diameter and the cavities inside were more or less equally well formed in both the portions (Plate IV, Fig. 1) but it was enclosed by a single leafsheath. At III/0 bifurcation was over and the two resultant arms were complete in all details and grew independently of each other to the end. The nodes and internodes henceforward were encased in distinct leaf-sheaths without any organic connection between them, and were smoothly reniform in outline for some distance. In the case of IV, the process of splitting was more or less the same except that it was shortened by one internode and that there was some modification of details. IV/ – 3 and IV/ – ii were normal in all respects while IV/ – 2 had a shallow depression on the side of the bud and was somewhat flattened on the other side. The main cavity was pushed to one side and another, smaller in size, was making its appearance.

At IV/ – i the bud doubled itself and a cleft appeared on the front side of the internode IV/ – 1 while that from IV/ – 2 was continued at the other end of the median diameter and the two cavities became more or less equal in size (Plate IV, Fig. 2). At IV/0 the rupture was complete and henceforward the two shoots so formed maintained their separate identities, remaining reniform for some distance as already mentioned for III.

In the case of II belonging to Co 213 it appeared that its bifurcation had been brought about by an injury probably caused by an insect at II/ – i and II/ – 1. The major portion of the injury (not shown in the photograph) was situated in A on the other side of the internode. On the bud side also the internode was slightly damaged in B (Plate III *a*). As a result, probably of unequal damage to the two halves of the internode, its cross-section, normally oval in outline, was flattened and became pyriform, the narrow and corresponding to the more injured half, *i.e.*, A. At II/0 there was a single bud situated in A and the root zone and the growth ring did not show any cleft and both II/ + 1A and II/ + 1B were enclosed in a single sheath. In the internodal region just above the growth ring, the stalk bifurcated abruptly into two arms, without any inkling of the ensuing split such as a fissure or cleft on the surface of the lower internodes or of development of two cavities inside them. The two resultant arms were unequal to start with, the thinner arm A corresponding to the more injured left half and the initial difference in the size was maintained throughout.

From the foregoing description of the three stalks, it would appear that the proximity and potency of exciting factor would determine the relative size of the arms. If the disturbing influence was slow to act and situated at considerable distance, the two arms would be equal in length and girth of their internodes as was the case in the two stalks of *Saraitha*. If on the other hand, the causative agency was nearby as in Co 213 and violently active, division would be abrupt and the resulting arms of different sizes.

(*b*) *Pseudo-fission*.—The splitting of the stalk of B.O. 17 was the slowest of all. It took four internodes to complete the fission and that too not for good, because after three internodes the two arms appeared to have coalesced to form a single stalk. If the actual length required to effect the rupture was taken into consideration, it was about 21 cm. in this case as against 14 cm. in stalk III. No injury or any other disturbing factor was found to exist on nodes and internodes even as far down as I/ – 5. The earliest external indication of the ensuing bifurcation appeared on internode I/ – 4 in which a fissure was found to have developed to the right of the bud on I/ – iv, there being no sign whatsoever on the other side. In I/ – 3 a similar

cleft was formed again on the right of the bud, while the one of I/− 4 was continued, in this internode as well and became more pronounced. At I/− ii and I/− i there was only one bud having been pushed further to the left of the cleavage. In the I/− 2, both the furrows deepened but not much. It was I/− 1 in which actual splitting took place though not completely because the two arms A and B remained organically connected by a thin parenchymatous strip (Plate VI, Figs. 1 and 2) which was absent at I/0. At this node a rudimentary bud was represented by a few scales on A, there being no corresponding structure on B. The two arms were independent of each other above I/0 and although they were encased henceforward in sheaths of their own, they were not identical, which fact would be discussed later in detail. A formed three distinct internodes and developed, at I/+ iiA, a small bud situated in a plane more or less at right angles to that in which the bud at I/− iv was formed. B consisted of only two internodes and was without any bud. Internode corresponding to I/+ 3A was represented at the upper end of I/+ 2B by a slight swelling, which was not demarcated into various parts of node and internode, there being no leaf and leaf scar corresponding to those of arm A at I/+ iiA.

Apparent coalescence of the two arms which started at approximately half the length of I/+3A, was over in about 3/4 cm. and resulted in the formation of a single node I/+ iii complete in all details and with a bud on B side. It was formed more or less in the same plane in which I/+ iiA developed it, or in other words at right angles to that of the bud at I/− iv. I/+ 4 did not bear any sign of having undergone a bifurcation except that it had a long shallow furrow (not shown in the photograph) situated laterally with respect to its own orientation. The furrow when considered in conjunction with the original axes of the stalk prevailing before splitting, would appear in its median plane. The axes of the stalk had therefore, rotated by about a right angle during bifurcation.

It would thus appear that the splitting of the stalk of B.O. 17 was abnormal not only because the two arms appeared to have coalesced after having maintained their independent identities for about 9 cm. but also because the two arms were not structurally identical with each other as was the case in the two stalks of *Saraitha* and one of Co 213. The adjacent surface of the two arms in B.O. 17 stalk did not develop root eyes and were like two pieces of jigsaw puzzle in broad outline the projections of one fitting into the depressions of the other, whereas in normal bifurcation the two arms were smoothly flattened on the adjacent sides on which root eyes were present as usual.

IV. Anatomy

(*a*) *Normal bifurcation*: (i) *Without any visible injury.*—This type of bifurcation did not interfere with the internal structure of the stalk to any great extent. The anatomical make up of IV/−3 and III/−4, III/−3 and III/−2 was perfectly normal except that another cavity had appeared in the last named internode (Plate IV, Fig. 1). Although none of the malformations associated with top borer attacks (Khanna and Sharma, 1948) or any other kind of disturbance in the internal structure was noticeable, the parenchymatous cells in the peripheral zone at the two ends of the median diameter along which fission would take place, were found to contain varying amounts of the brown substance apparently similar to that noted before (*loc. cit.*). The vascular bundles however, even in this portion of matrix were not clogged. In IV/−2 at one end of the plane of fission having a groove, an equilaterally triangular patch of comparatively thin-walled parenchyma (Plate IV, Fig. 3 *p*) appeared in between the outermost vascular bundles and a highly lignified zone of small celled parenchyma (called henceforward the hypodermal zone) and was continued taperingly on either side of the depression. It was covered over by epidermis except in the depression where it having atrophied, the outermost cells of hypodermal zone also showed necrosis. The parenchymatous ground tissues in the neighbourhood had heavy deposit of the brown substance and although xylem vessels of the vascular bundles embedded in this matrix were not choked, phlœm was sometimes affected. This variation in the anatomical make-up was continued in IV/−1 as well without any change in size or structure. In the case of III/−1 the parenchymatous patch was cresentiform instead of being triangular and was continued on either side of the depression in which another layer of this tissue (Plate IV, Fig. 5 *p'*) was present above the hypodermal zone showing necrosis.

The other end of the median plane of IV/−2 did not show any depression although the parenchymatous cells forming the matrix contained the same brown substance. Higher up in IV/−1, a comparatively thin hypodermal zone (similar to that shown in Plate IV, Fig. 4) was present in the depression, where necrosis having set in, epidermis was absent. But the triangular patch of parenchyma was absent at this end. The hypodermal zone was continued as mentioned before taperingly on either side of the depression beyond which it was covered over by epidermis. In the case of III/−1 (Plate IV, Fig. 4), the depression being shallow, the epidermis was intact but the structure otherwise was the same as that described above. The brown substance also was present in the matrix but the vascular bundles

were mostly free of it. It would, therefore, appear that although the plane of fission was clearly laid down in III/ − 1 and IV/ − 1 it did not disturb the internal structure to any appreciable extent except that both of them had two cavities each. In the case of III/ − 1, however, lignification of parenchyma along the plane of fission was comparatively poor for some distance from both the ends (Plate IV, Fig. 5 *I*).

Above the node 0, the structure of the two arms was in both the stalks normal in all respects but for very light deposit of the brown substance in parenchymatous cells in the peripheral zone in adjacent flattened portions of the internodes. Since these and internodes above them did not show any outward sign of disturbed anatomy, they were not studied any further.

(ii) *With visible injury.*—In the case of Co 213 stalk, II/ − 2 and II/ − 1 were normal in their anatomical structure although the latter along with the node II/ − i having been injured, deformed out of its normal oval cross-section. The injured tissue of II/ − 1 showed strructure resembling Plate III, Figs. 3 and 4 (Khanna and Sharma, 1948) which indicated that the internode was fairly well advanced towards maturation when it was attacked (Plate IV, Fig. 6). II/ − 1 had no signs of the ensuing split as the plane of splitting was not demarcated by the formation of grooves externally or internally by the appearance of hypodermal zone or the presence of parenchymatous proliferation or necrosis of the epidermis. The ground tissue also was uniformly lignified throughout the whole cross-section. In the front fissure above II/0 there was no necrosis but an outgrowth consisting of hypodermal zone and a layer of morbid parenchymatous tissue was present (Plate III, Fig. 7). Both of them were continued on the either side and were covered over by the epidermis. Parenchymatous cells of general ground tissue contained granular substance which stained deeply, and was different from the brown substance referred to before. The intensity of deposit in cells decreased as one moved farther away both horizontally towards the centre or sideways and vertically from the node. Very light deposit of the brown substance was present in cells on the fringe of parenchyma having this granular substance. None of the elements of vascular bundles was clogged. The plane of fission was demarcated along the median diameter by parenchymatous cells in appearance squarish or oblong in cross-section, and by vascular bundles having been cut slatingly. At the other end of the diameter there was no structural variation.

In II/ + 1A and II/ + 1B, the injured portions resembled Plate IV, Fig. 6, while other portions of II/ + 1A had a thin layer of hypodermal zone and broad layer of morbid parenchyma in which sparsely scattered vascular

bundles were found (Plate IV, Fig. 8). Parenchyma forming the ground tissue had brown deposit but the vascular bundles embedded in it were free of it. The rind was normally constituted in uninjured portions of II/+ 1B.

Internodes higher up namely II/+ 2A and II/+ 2B were normal in all respects. Even the brown deposit so characteristic of recently disturbed anatomy was absent in their adjacent areas.

(*b*) *Pseudo-fission.*—Abnormal bifurcation in the B.O. 17 stalk was totally different from the other three cases so for described. Apart from the morphological differences already given in the preceding section, the antomy of this stalk was very much affected and over a considerable distance. I/− 5 which had no external indication such as a cleft and in which the rind was normally constituted, had one big and two small patches of small-celled parenchyma arranged along the median diameter near the natural cavity of the cane (Plate V, Fig. 1). The vascular bundles in the neighbourhood of these patches were sometimes deformed but were not clogged. Brown substance was present only towards the periphery. It was quite probable that the disturbance in the storage tissue started in I/− 6 or in still lower internodes. But where it actually began could not be stated definitely as internodes below I/− 5 were not preserved and were, therefore, not available for study. In I/− 4 the disturbed area increased in size and progressed towards the rind where a cleft and a hypodermal zone had appeared. Within the disturbed area, small-celled parenchyma contained a patch consisting of dead cells (Plate V, Fig. 2) or a cavity partly full of decaying cells (Plate III, Fig. 3). Phlœm of some vascular bundles was affected and parenchymatous cells were full of the brown substance. In I/− 3 the plane of fission which had been fixed externally by the presence of fissures in front and back, was discernible in the internal structure also, as small patches of small-celled parenchyma were present in the distal half in which a new and somewhat shallow cleft had appeared (Plate V, Fig. 4). The natural cavity was absent and that formed by the disorganisation of diseased tissue though well defined in the lower half of the internode, was slowly obliterated by the squeezing of small-celled parenchyma brought about by the growth of cells on its periphery. Phlœm of neighbouring vascular bundles and parenchymatous cells below the hypodermal zone contained varying quantities of the brown substance. The plane of longitudinal fission in I/− 2 was well defined by a chain of patches of small- and elongated-celled parenchyma, and small cavities enclosed in them and was slowly perfected as one approached I/− i (Plate V, Figs. 5 and 6). The rind was interrupted only in the fissures. At one end of the median plane a wedge of thin-walled parenchyma full of protoplasmic substance was present and

was continued on either side of the depression between the hypodermal zone and vascular bundles. The intensity of the cell contents decreased as one moved away from the depression. At places cavities surrounded by necrosis were found in the longitudinal depression. A and B arms of I/− 1 were connected by a narrow strip formed by thin-walled elongated parenchymatous cells (Plate VI, Figs. 1 and 2). Epidermis and vascular bundles typically peripheral* in their organization were present up to the points marked *a*, *b*, *c* and *d* in Plate VI, Fig. 1. The tissues between these points were covered over by heavily lignified cells, and the vascular bundles were central in structure. Necrosis and variations in the structure of parenchymatous ground tissues did not follow any particular plan or pattern. Curiously enough the vascular bundles and cells of the ground tissue near the fissure were mostly free of all foreign substances. Only near the origin of the fissures the latter contained brown substance.

Above I/0, the two arms on adjacent sides had no properly constituted rind (Plate VI, Figs. 3 and 4). There was no epidermis and the vascular bundles were practically absent. The outermost tissue mostly consisted of lignified small-celled parenchyma. An island of seven vascular bundles along with a thick band of highly lignified small-celled parenchyma (Plate VI, Fig. 3) appeared to be a crude attempt on the part of I/+ 1A to form a rind. The outlines of both the arms on adjacent sides were not smooth as was the case of in the three stalks already described. In higher internodes I/+ 2A and 1/+ 2B more typically in the latter (Plate VI, Figs. 5 and 6), a rind consisting of epidermis, a broad highly lignified hypodermal zone and peripherally constituted vascular bundles, was formed over the greater part of the adjacent sides. The upper swollen portion of I/+ 2B had vascular bundles characteristic of a node (Plate VI, Fig. 7) while in I/+ 3A they were internodal in structure. In the region of pseudo-coalescence (Plate VII, Figs. 1–5) vascular bundles of the two arms maintained their respective nature, *i.e.*, in the portion corresponding to A, they were internodal and interspersed all over the ground tissue while in that corresponding to B they were nodal in structure and were crowded together right up to the edge of the connecting tissue. Vascular bundles from the nodal half of the cross-section appeared to pass into the internodal half and were cut longitudinally (Plate VII, Figs. 2–4). The parenchymatous cells forming the connecting tissue were also squarish or oblong in transverse section indicating thereby that their longitudinal axis was more or less at right angles to that of the

* The sheath at its xylem end in vascular bundles of the peripheral region is much more massive than at the phlœm end. In the vascular bundles of the central region the sheath is equally thick at both the ends.

stalk (Plate VII, Figs. 1–5 and 6). The connecting tissue slowly increased in breadth and the character of vascular bundles in B portion changed from nodal to internodal (Plate VII, *cf.* Figs. 1 and 5) as one approached I/+ iii. In I/+ 4 there was no sign of the stalk having undergone a fission except that near the depression, the cells of the ground tissue contained heavy deposits of brown substance (Plate VII, Fig. 7).

V. Discussion

From the foregoing morphological description and anatomical details of the four stalks, it would appear that they fell into two distinct groups, namely:

(i) Co 213 and *Saraitha* (both the stalks) in which as a result of longitudinal fission, the resulting arms grew independently thenceforward and were identical and complete in themselves, each node in both the arms having a bud. In all the three cases the adjoining surfaces of the two arms were smooth and with root eyes in root zone and the internal structure was quite normal. That the two arms in Co 213 stalk were different from each other in the size of the internodes was clearly due to the position of the injury.

(ii) B.O. 17 stalk in which the longitudinal fission was not permanent as the two arms appeared to have coalesced after remaining separate for about 9 cm. In arm A there were three distinct internodes with buds on two of the nodes, *i.e.*, I/0A and I/+ iiA. The other arm B had only two internodes with a swelling, nodal in structure at its upper end. There was no bud at its nodes. The adjoining surfaces of the two arms were not smooth and without root eyes in root zone and there was no properly constituted rind in those portions. The epidermis was replaced by lignified parenchymatous tissue having very few vascular bundles.

The reason for the splitting of Co 213 stalk was evident because in II/– 1 there was a distinct insect-injury in causing which some of the growth-promoting substances called auxins were incidentally introduced into plant tissues, and the growing point being very near, it broke up into two. Martin (1938) by artificially inoculating the extract of green leaf-hopper of sugarcane, *Dræculacephala mollipes* (family Jassidæ) was able to break up the apical bud into several buds in two out of 21 stalks of P.O.J. 2878.

The case of stalks of *Saraitha* was slightly on a different footing. Although no injury or any other kind of disturbing agency was present within 3–4 internodes below the node of actual division, some factor must be working to bring about the doubling of buds, the appearance and development of another cavity, and retarding the lignification of parenchymatous cells along a particular plane. Evidently it was slow in action as compared to

that of Co 213, but was fast enough to overtake the growing point and of sufficient intensity to split it into two more or less equal arms. The mode and stages of bifurcation along with the concurrent teratological condition and the final outcome of the whole process therefore pointed to the presence of a disturbing agency at a considerable distance from the actual seat of growth activity. From the resultant effect of this substance, it might be taken to be similar or very much allied to that of Co 213.

In the stalk of B.O. 17 the disturbance in the internal structure appeared to have been brought about by a substance of a totally different nature, because instead of inciting the cells to proliferate, it arrested their growth in the first instance and then brought about their death. The dead cells disintegrated yielding their place to a cavity to fill which surrounding parenchymatous cells in certain cases elongated to form a kind of palisade-like tissue.

In I/ − 5 the affected area being very small, it appeared that the growth arresting substance was just beginning to manifest its presence. In higher internodes, progressively greater area of parenchymatous ground tissue along the median diameter was brought under its influence till in I/ − 1 necrosis along that diameter was widespread, and more or less continuous from front to back resulting in complete rupture at I/0, and the presence of elongated cells in I/ − 1 was due to a subsequent reaction of the surrounding tissues. The presence of two arms higher up with their jig-saw-like adjoining surfaces without properly constituted rind in these portions in internodal region, and without root eyes in the nodal region, pointed to a splitting totally different from that undergone by the other three stalks. The appearance of a bud on alternate nodes (I/0A and I/ + iiA) further strengthened this view. It seems that the bud at I/ + iB and the internode I/ + 3B could not be formed probably due to too much physiological strain though there seemed to be an attempt at the formation of the latter as was apparent from the change of character of vascular bundles from nodal to internodal in the pseudo-joint. The disturbing agency ceased to function abruptly and the I/ + 4 showed no sign of any stress except that it was flattened very much with respect to the original orientation of the stalk. Whether the disturbing element exhausted itself or a change took place in environment could not be stated definitely. But this much might be said that the stalk did not react so as to stop the rot.

It would, therefore, appear that B.O. 17 stalk was a case of pseudo-fission brought about by the arrest of growth of and later on the death of parenchymatous cells along the median plane while in Co 213 and *Saraitha* stalks, fission was brought about by the splitting of the growing point and that the

agency causing fission and pseudo-fission were fundamentally different in nature. It is however, interesting to note that both of them worked in the same plane, *i.e.*, along the median diameter which seemed to occupy a position unique in itself which view was further supported by the fact that once it was laid down, buds would always be formed in that and in no other plane. The shifting of bud slowly through nearly a right angle in B.O. 17 might be interpreted as an attempt on the part of the stalk to preserve this unique diameter the fundamental use of which to the stalk is still to be fully comprehended.

VI. Summary

1. Of the four stalks described from morphological and anatomical points of view, two of *Saraitha* and one of Co 213 showed fission and that of B.O. 17 pseudo-fission.

2. In Co 213 in which the internode just below the node of bifurcation bore signs of insect-injury, had unequal arms and the reaction of tissues was similar to that described elsewhere. As fission was violent and swift in this case, the arms were unequal in dimensions.

3. In the stalks of *Saraitha* there was no visible injury or any other disturbance 3–4 internodes below the node of actual fission. Slow changes such as appearance of two cavities, poor lignification of parenchyma along the median plane, doubling of buds and formation of more or less equal arms appeared to indicate the presence of a disturbing factor at considerable distance from the seat of growth activity.

4. In Co 213 and the *Saraitha*, bifurcation of stem was brought about by the fission of growing point itself, by the action of auxins or similar growth-promoting substances.

5. In B.O. 17 splitting was brought about by the necrosis of parenchymatous cells along the median plane after their growth had been arrested by a slow acting factor. The absence of properly constituted rind in the adjoining portions of the two arms and presence of a bud on alternate nodes on one of the arms supported this view. B.O. 17 was therefore, a case of pseudofission.

6. The disturbing element in B.O. 17 was different from that working in the other two varieties. The former arrested the growth of cells and ultimately brought about their disintegration while the latter incited the cells to proliferation.

7. That both the kinds of substances worked along the median diameter showed that it was different from all other diameters and occupied a unique

position in the organisation of sugarcane stalk the full significance of which is still imperfectly understood.

VII. Acknowledgements

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References

1. Khanna, K. L., and Sharma, S. L. "Anatomy of sugarcane stalk as influenced by top borer (*Scirphophaga nivella* F.) attack," *Proc. Ind. Acad. Sci.*, 1948, **27**, 1–3.

2. Martin, J. P. .. "Stem galls of sugarcane induced with an insect extract," *Proc. VI Cong. Int. Soc. Sug. Tcc.*, 1938, 461–67.

Explanation of Plates

Plate III

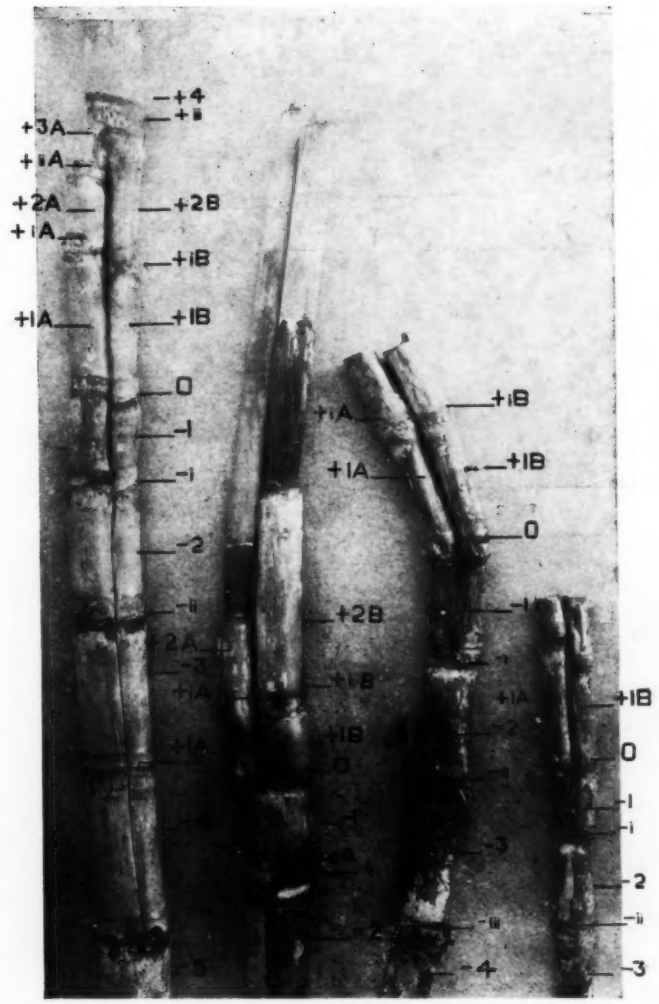
Photograph of the four stems belonging to, from left to right, B. 0·17 (I), Co 213 (II) and *Saraitha* (III and IV). The initial difference in the size of internodes forming the two arms in II was maintained while those of III and IV were equal for considerable distance. There was doubling of buds in the latter two stalks before fission while the bud on II/−i was destroyed by injury (*a*). All nodes in the three stalks (II–IV) had a bud each after splitting. But in I it was formed on alternate nodes (I/0A and I/+iiA) on arm A only (5/6 natural size).

Plate IV

Transverse sections of stalks II, III and IV, showing normal fission. Figs. 1, 4 and 5, III/−1; Figs. 2 and 3, IV/−1 ; Fig. 6, II/−1 ; Fig. 7, just above II/0 ; Fig. 8, II/+1A. Figs. 1 and 2 show two cavities (C) formed prior to bifurcation. In Figs. 3 and 5 patches of small-celled parenchyma (*p*) covered over by hypodermal zone (*h*) are illustrated ; in Fig. 5, there is another layer of the former (*p'*) over the latter. The hypodermal layer (*h*) in Fig. 4 is narrow. That the plane of fission has already been decided upon is indicated by the poor lignification of ground tissue (*l*). Fig. 6 shows insect-injury to tissues fairly well advanced towards maturation. In Fig. 7 there is no necrosis in the fissure which is occupied by an outgrowth, consisting of small-celled parenchyma and hypodermal zone. A broad zone of morbid parenchyma having sparsely scattered vascular bundles is shown in Fig. 8 (Figs. 1 and 6, ×10 ; Figs. 2 and 5, ×15 ; Fig. 3, ×50 ; Fig. 4, ×100 ; Figs. 7 and 8, ×25. Opaque areas in this plate and in subsequent ones are due to either sections being very thick or air bubbles getting into them).

Plate V

Transverse sections of the stem of B. 0·17 showing progress of pseudo-fission. Figs. 1, 2 and 3 illustrate small-celled parenchymatous patches showing the arrest of growth. Secondary cavities (*c*) are enclosed within them. In Fig. 2 one of them (*c'*) is full of dead-cells which have disintegrated to some extent in Fig. 3. Formation of a fissure and the interruption of the rind are illustrated in Fig. 4. Small-celled ground tissue at the bottom of the cleft may be noted. Figs. 5 and 6 when joined together at X and X' give the entire plane of fission as it appears near I/−i. There is lateral inversion in Fig. 6 : that is why X' is upside down image of X. Along the plane of fission are situated patches of morbid parenchyma and secondary cavities (Fig. 1, ×20 ; Figs. 2, 4, 5 and 6, ×10 ; and Fig. 3, ×15).



1 2 3 4

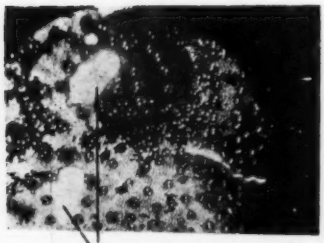


FIG. 1

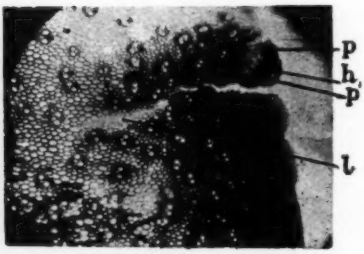


FIG.5

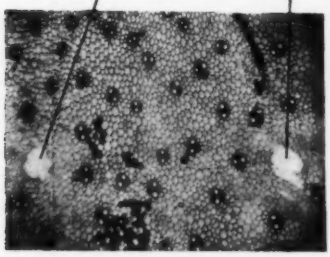


FIG.2

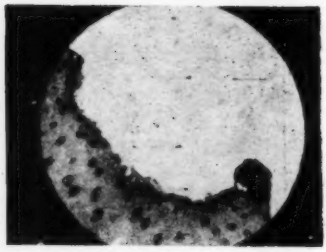


FIG.6

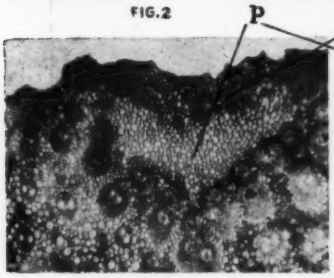
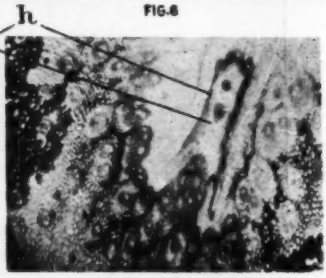


FIG.3



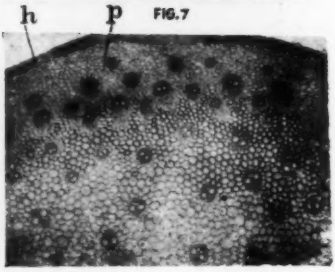


FIG.7

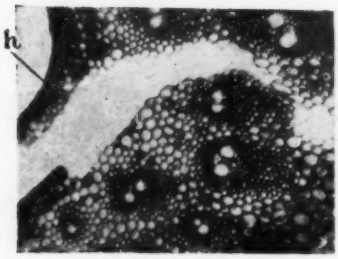


FIG.4

FIG.8

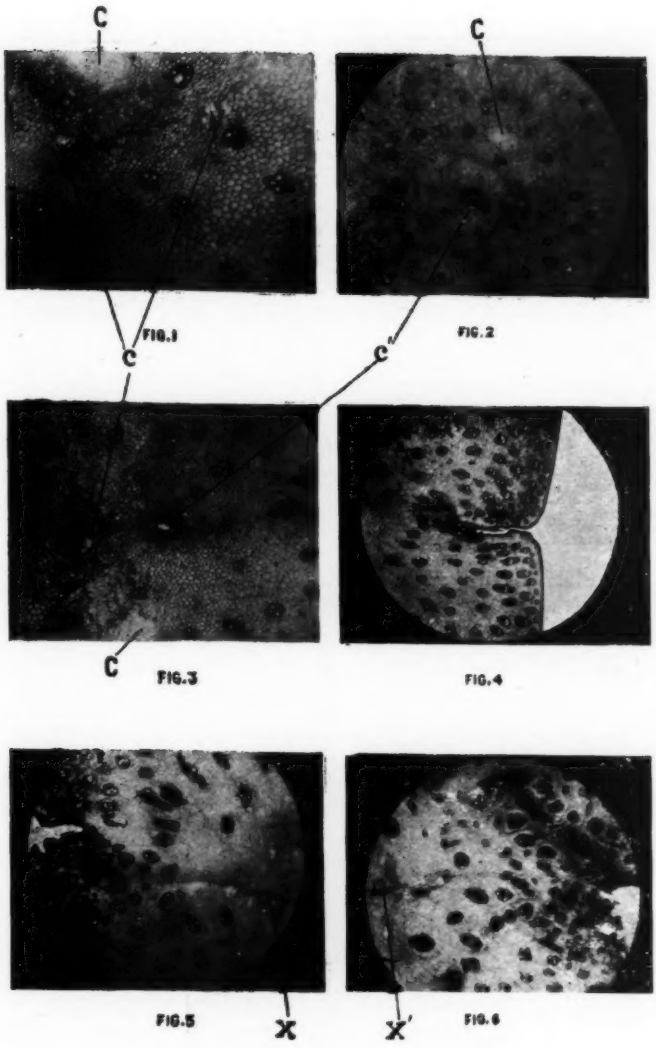


FIG. 1 FIG. 2

FIG. 3 FIG. 4

FIG. 5 FIG. 6

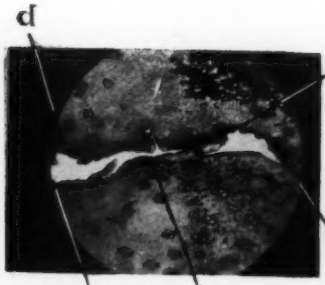


FIG. 1

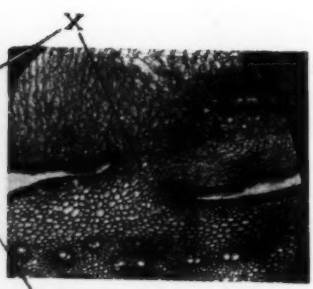


FIG. 2

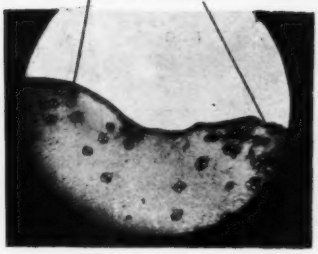


FIG. 3

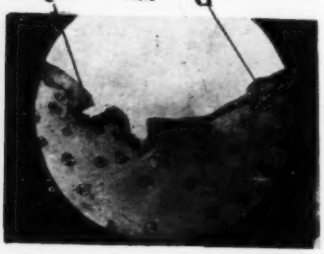


FIG. 4

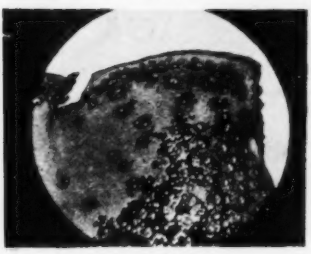


FIG. 5

FIG. 6

FIG. 7

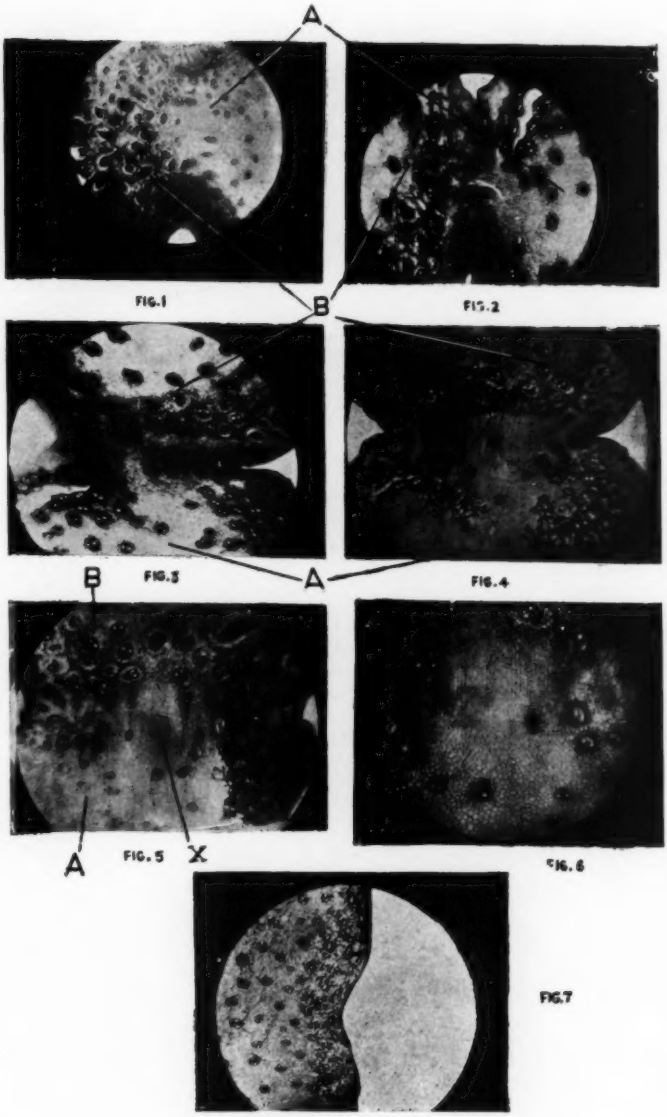

A
B
FIG. 1
FIG. 2
B
FIG. 3
A
FIG. 4
B
A
FIG. 5
X
FIG. 6
FIG. 7

PLATE VI

Transverse sections of the two arms of B. O. 17 stem. The two arms of I/−1 are still connected with each other by a narrow strip of morbid parenchyma (X) in Figs. 1 and 2. Between *a* and *b*, *c* and *d* in Fig. 1, and Figs. 3 and 4, there is no properly constituted rind. The tissues are covered over by highly lignified zone of morbid parenchyma. Vascular bundles between these points are few and far between and are central in nature. Figs. 5 and 6 show attempts at the formation of rind which are more successful in the latter. The nodal character of the swollen upper end of I/+2B is apparent because vascular bundles are cut in all planes and the parenchymatous cells surrounding them are arranged stellate fashion (Figs. 1, 3, 4, 5 and 6, ×10; Fig. 2, ×65; Fig. 7, ×20).

PLATE VII

Transverse sections of tne pseudo-joint at various levels from below upwards and I/+4. Figs. 1–5 illustrate the internodal and nodal character of the two portions corresponding to arms A and B. In portion A, the vascular bundles are scattered in the ground tissue while in B, they are crowded together right up to tne edge of the connective tissue (X). Their dispersal and the change from nodal to internodal character are noteworthy in Fig. 5. They are cut longitudinally while passing through the connective tissue as shown in Figs. 2–4. The parenchymatous cells in this region are also cut in the same way. Fig. 6 is an enlargement of part of Fig. 5 to illustrate the point. Fig. 7 passes through I/+4 which is normal in structure (Figs. 1–5, ×5; Fig. 6, ×15; Fig. 7, ×10).

ADDITIONS TO FUNGI OF MADRAS—VI

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(42) *Goplana indica* Ramakrishnan, T. S. and K., sp. nov.

Telia only known; spots amphigenous, brown, up to 3 cm. in diam.; *telia* hypophyllous, subepidermal, erumpent, waxy, gregarious, bright orange when young, turning dark brown with age; *teliospores* sessile, one celled, club-shaped, produced in pairs on basal cells, contents orange, wall thin and gelatinising when moistened, $52 \times 13\,\mu$ ($44–72 \times 8–20\,\mu$), germinating at once by an internal promycelium; sterigmata stout; basidiospores spherical.

On living leaves of *Beilschmiedia roxburghiana* Nees., Chinnakallar, Anamalais, 4-2-1948, T. S. Ramakrishnan and K. Ramakrishnan.

Telia tantum nota; maculis amphigenis, brunneis, 3 cm. diam.; teliis hypophyllis, subepidermalibus, erumpentibus, cereis, gregariis, tenera aetate fulgido luteo colore, provecta aetate fusco-brunneo colore; *teliosporis* sessilibus, unicellularibus, clavatis, binis ex cellulis inferis prodeuntibus contentis luteo colore, pariete levi, gelatinoso $52 \times 13\,\mu$ ($44–72 \times 8–20\,\mu$) statim germinantibus; promycelio interno, sterigmatibus pinguibus; basidiosporis rotundis.

In foliis vivis *Beilschmeidiæ roxburghianæ* Nees., Chinnakallar, Anamalais, 4-2-1948, T. S. Ramakrishnan et K. Ramakrishnan.

Fig. 1. *Goplana indica*—a pair of teliospores (one germinating) (× 325)

This interesting rust belongs to a genus not previously recorded from India. The telia are very crowded, conspicuously waxy, and prominent

from the brilliant orange colour in the early stages. When the telia are teased out it is seen that a number of orange coloured club shaped cells are present at the base which give rise to apical clusters of secondary and tertiary sporogenous cells. The terminal cells give rise to two teliospores each which may be of different ages. On germination, the teliospores divide into five cells of which the lowermost is sterile. The four fertile cells produce one basidiospore each, on long thick sterigmata. Meanwhile the wall of the teliospore becomes gelatinised and all the spores stick together embedded in the gelatinous matrix.

Four species of this genus have been recorded, three from Java (Saccardo, 1902 and 1912) and one from Samoa and the Philippines (Cummins, 1935). The rust under study differs from these in spore size, and its host plant.

(43) *Chrysocelis indica* Ramakrishnan, T. S. and K., sp. nov.

Telia only present, hypophyllous, forming bright golden yellow patches covering large areas of the leaf surface, subepidermal; *teliospore* one celled cylindric to clavate, thin walled, orange yellow in colour, sessile, 30 × 8 μ (24–40 × 6–12 μ), germinating immediately *in situ*; basidium light coloured, sporidia globular or oval.

On living leaves of *Justicia betonica* L., Burliar, Nilgiris, 24-1-1948, T. S. Ramakrishnan and K. Ramakrishnan.

Telia tantum nota, soris teleutosporiferis hypophyllis, fulgido aureo flavo colore, dense gregariis, sæpe totam fere inferiorem foliorum superficiem occupantibus; *teleutosporis* unicellularibus, cylindricis vel clavatis, sessilibus, membranis hyalinis tenuissimis, 30 × 8μ (24–40 × 6–12 μ), *in situ* statim germinantibus, contentis luteo colore; basidiis pallidis; sporidiis globosis vel ovalibus.

In vivis foliis *Justiciæ betonicæ* L., Burliar, Nilgiris, 24-1-1948, T. S. Ramakrishnan et K. Ramakrishnan.

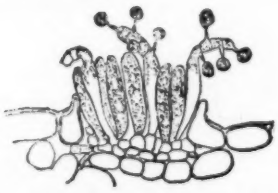


Fig. 2. *Chrysocelis indica*—section through telium (× 200)

This rust presents a striking appearance forming continuous golden yellow patches on the lower surface of the leaves. With age the colour becomes lighter. The upper surface is very much puckered and pale green

in colour. The telia develop in the substomatal air spaces. There is a compact stroma of one or two layers of fungal cells. From the upper layer of this stroma groups of sessile teliospores are produced. These protrude through the stomatal pore pushing the epidermal cells apart. The germination is almost immediate and owing to the formation of large numbers of basidia the surface of the leaf has a velvetty appearance.

Chnoopsora butleri Diet and Syd. has been recorded on *Adathoda vasika* (*Justicia adathoda*) (Dietel, 1906). The rust under study has free sessile teliospores and thus is a *Chrysocelis*. It differs from *Chnoopsora* in not having a crustaceous sorus.

(44) *Cronartium fici* Ramakrishnan, T. S. and K., sp. nov.

Pycnia and *aecia* not known; *uredia* hypophyllous, minute, scattered, subepidermal, covered by an evanescent peridium; *urediospores* subglobose or elliptic, to angular, echinulate, apex slightly thickened and darker coloured, 27 × 20 μ (20–36 × 16–24 μ), short stalked; *telia* hypophyllous, filiform, up to 5 mm. in length, dark brown, subepidermal in origin; *teliospores* closely united, oblong, 35 × 9μ (20–48 × 6–16 μ), wall smooth, germinating immediately; basidia short, lateral, 4 celled, basidiospore spherical.

On living leaves of *Ficus* sp., Burliar, Nilgiris, 24–1–1918, T. S. Ramakrishnan and K. Ramakrishnan.

Pycnia et *aecia* non cognita; *uredosoris* hypophyllis, minutis, sparsis, peridio evanescente coopertis; *uredosporis* subglobosis, ellipticis vel

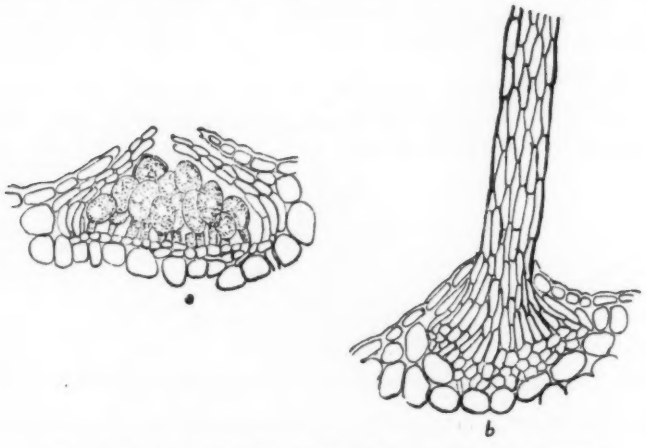


FIG. 3 *a*. *Cronartium fici*—Section through uredium (× 200)
FIG. 3 *b*. Telium (× 300)

angulatis, echinulatis, breviter pedicellatis, apice leniter incrassatis; *teleutosoris* hypophyllis, filiformibus, rectis vel curvatis, usque 5 mm. longis, fusco-brunneo colore; *teleutosporis* unilocularibus, arcte cohaerentibus, oblongis, membranis levibus, statim germinantibus; basidiis brevibus, laeteralibus, 4-cellularibus; sporidis globosis.

In vivis foliis *Fici* sp., Burliar, Nilgiris, 24–1–1948, T. S. Ramakrishnan et K. Ramakrishnan.

Crossopsora fici Arth. and Cumm. has been recorded on *Ficus capensis* from Uganda and on *F. variegata* from the Philippines (Cummins, 1945). But the rust under study is not a *Crossopsora*, as the uredia possess peridia and are not surrounded by paraphyses.

(45) *Melampsora stereospermi*, Ramakrishnan, T. S. and K. sp. nov.

Spots circular, amphigenous, hypertrophied, of varying sizes, dark brown; *pycnia* minute, black, amphigenous, interspersed with the uredia, subcuticular, hemispherical, paraphysate; *uredia* amphigenous, mostly hypophyllous, subcuticular, arising from a plectenchymatous stroma, pulvinate, hazel coloured, paraphysate; *urediospores* pedicellate, subglobose to irregular, inner wall brown, outer wall thick hyaline, gelatinising, spinulose, inner measurements 23 × 18 μ (16–28 × 16–24 μ) outer measurements 31 × 30 μ (28–36 × 28–36 μ), pedicel hyaline; germ pores two, subequatorial.

Telial spots amphigenous, chocolate brown; *telia* hypophyllous, circular, scattered, subcuticular, in one layered crusts; *teliospores* sessile, one celled, cylindric, laterally closely united, 45 × 8μ (36–52 × 4–12 μ), occasionally transversely divided, subhyaline.

On living leaves of *Stereospermum tetragonum* DC., Gudalur, Nilgiris, 21–5–1947, T. S. Ramakrishnan and T. V. Subramanian; Walayar, Malabar, 7–1–1948, T. S. Ramakrishnan and K. Ramakrishnan, (type) and *S. suaveolens*, DC., Walayar, Malabar, 7–1–1948, T. S. Ramakrishnan and K. Ramakrishnan.

Maculis orbicularibus, amphigenis, fusco brunneis, amplificatis, diversis, amplituolinibus; *pycniis* minutis atris, amphigenis; ı re ii immixtis, subcuticularibus, hemisphericis, paraphysatis; soris uredosporiferis amphigenis, plerumque hypophyllis, subcuticularibus, pulvinatis, subfulvo colore paraphysatis; *uredosporis* subglobosis vel irregularibus, pedicellatis, fulvo pariete interiori; pariete exteriori pingui, hyalino, gelatinoso, spinuloso; mensuris interioribus 23 × 18 μ (16–28 × 16–24 μ), mensuris exterioribus 31 × 30 μ (28–36 × 28–36 μ); pedicellis hyalinis, poris germinationis duobus, subequatorialibus.

Maculis amphigenis; soris teleutosporiferis hypophyllis, orbicularibus, sparsis, subcuticularibus, crustiformibus; *teleutosporis* cylindricis, unicellularibus, sessilibus, arcte cohaerentibus, 45× 8 μ (36–52× 4–12 μ), subhyalinis, aliquando cum uno septo transverso.

In vivis foliis *Stereospermi tetragoni* DC., Gudalur, Nilgiris, 21–5–1947, T. S. Ramakrishnan et T. V. Subramanian; Walayar, Malabar, 7–1–1948, T. S. Ramakrishnan et K. Ramakrishnan, (typus) et *S. suaveolens* DC., Walayar, Malabar, 7–1–1948, T. S. Ramakrishnan et K. Ramakrishnan.

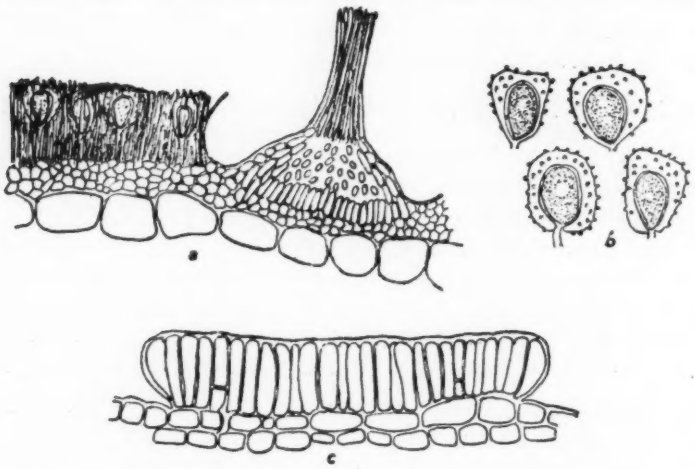


FIG. 4. *Melampsora stereospermi*—(*a*) section through uredium and pycnium (×125) (*b*) urediospores (× 250), (*c*) section through telium (× 200)

Infections are first seen as small circular brown spots in which the pycnia are prominent. Later the spots enlarge and the tissue becomes hypertrophied. A convex bulge forms towards the lower surface and a corresponding depression on the upper surface. By this time numerous uredia have been formed mostly on the lower surface giving a hazel colour to the spots. The pycnia stand out as black dots in the midst of the uredia. The uredia form compact round sori often coalescing. A multilayered stroma is developed under the cuticle and from this the urediospores project, rupturing the cuticle. The hyphæ of the fungus are intercellular. They send in peculiar clustered haustoria into the mesophyll cells. The urediospores are pedicellate and often formed in clusters. The wall of the spore is bilamellate, the inner brown and the outer hyaline and irregularly thickened. The outer wall is thinnest at the base. Hyaline, filiform, thin-walled paraphyses are seen mixed with the urediospores. A peridium is not

evident. Two germpores can be seen in the inner wall in the lower half of the spore.

The telia develop as circular, depressed, shining, definite crusts having slightly raised margins. The colour deepens with age. Finally the entire spot bearing the telium falls off leaving shot holes. The teliospores are closely packed, sessile, laterally united and form one layered compact crusts. Sometimes a partition wall develops in the spore dividing it into two cells, the lower cell being much smaller.

The telial characters suggest that the rust is a *Melampsora*. Mundkur and Thirumalachar (1945) have described *Mehtamyces stereospermi* on *Stereospermum suaveolens*. The urediospores of the two rusts are very much alike. But in the present rust the uredia are subcuticular and are mixed with the pycnia, whereas in *Mehtamyces* the uredia are subepidermal and the pycnia have not been observed. The telia of *Mehtamyces* are in many layered crusts while in the present rust they form single layered crusts.

(46) *Puccinia jasminicola* Ramakrishnan, T. S. and K. sp. nov.

Spots amphigenous, irregular, pale yellowish green to light brown; *telia* hypophyllous, rarely amphigenous, minute, crowded, isolated, erumpent, chocolate brown; *teliospores* 2-celled, brown, constricted at the septum, 37 × 19 μ (25–44 × 14–25 μ) apex rounded or obtuse, thickened up to 9 μ, germpores one in each cell, pedicel hyaline, short; mesospores present, paraphyses rare, capitate.

On living leaves of *Jasminum flexile* Vahl., Burliar, Nilgiris, 22–7–1948, T. S. Ramakrishnan and K. Ramakrishnan.

Maculis amphigenis, irregularibus, pallide flavo-viridibus, vel subbrunneis; *teliis* hypophyllis, minutis, aggregatis, isolatis, erumpentibus, subepidermalibus; *teliosporis* bicellulatis, brunneis, medio constrictis,

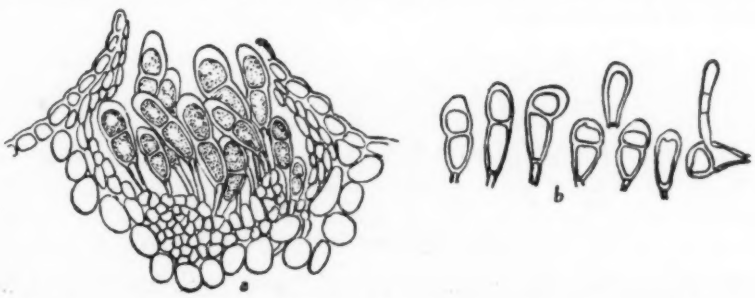


FIG. 5. *Puccinia jasminicola*—*a*. section through telium, *b*. teliospores (×200)

37 × 19 μ (25–44 × 14–25 μ), apice rotundatis vel obtusis, incrassatis usque 9 μ; pedicellis hyalinis, brevibus; mesosporis praesentibus, paraphysibus rariis, capitatis.

In vivis foliis *Jasmini flexile* Vahl., Burliar, Nilgiris, 22–7–1948, T. S. Ramakrishnan et K. Ramakrishnan.

Four species of *Puccinia* producing the telial stage have been recorded on *Jasminum* spp. namely *P. jasmini* DC., *P. exhauriens* Theum, *P. abyssinica* (P. Henn) Syd., and *P. Zimmermanniana* P. Henn., The rust under study has the telial stage only, on *Jasminum flexile*. The telia are amphigenous, isolated and deep seated. The remnants of the epidermis form a distinct ring round the telium. The spores have very short stalks. A few paraphyses with rounded thickened apices are seen in some of the telia. Mesospores are present.

The present rust is different from the others in the presence of the paraphyses and mesospores, in the amphigenous telia, short stalks of the spores and in the size of the teliospores.

(47) *Puccinia exhauriens* Theum

On leaves of *Jasminum pubescens* Willd., Cinchona, Anamalais, 4–2–1948, T. S. Ramakrishnan and K. Ramakrishnan.

This rust was noticed on the leaves of *Jasminum pubescens*, causing slight malformations. The spots are yellowish green on the upper side and on the convex lower surface numerous dark brown, pulverulent telia are seen arranged in circles. The telia are isolated when young but become confluent in older spots. The teliospores are very variable in shape and measure 47 × 16 μ (27–61 × 11–22 μ). The upper cell of the spore is shorter and broader. The apex is rounded or blunt, thickened up to 9 μ or quite thin. The stalks are very short. These characters more or less resemble those described for *P. exhauriens*.

FIG. 6. *Puccinia exhauriens*—Teliospores (× 325)

This species was first recorded on *Jasminum tortuosum* from Africa (Sydow, 1904).

(48) *Uredo malabarica* Ramakrishnan, T. S. and K. sp. nov.

Spots amphigenous, minute, limited by veinlets, isolated or confluent, angular, $\frac{1}{3} - \frac{1}{2}$ mm. diam.; yellowish brown; *uredia* hypophyllous, isolated or gregarious, erumpent, minute, subepidermal; *urediospores* variable in shape, prominently echinulate, $24 \times 16\,\mu$ ($20–28 \times 12–20\,\mu$); paraphyses numerous, clavate, subhyaline.

On living leaves of *Bridelia retusa* Spr., Walayar, Malabar, 7–1–1948, T. S. Ramakrishnan and K. Ramakrishnan.

Maculis amphigenis, minutis, isolatis, vel confluentibus, flavo brunneo colore, angularibus, venis delimitatis; $\frac{1}{3}-\frac{1}{2}$ mm. diam.; soris *uredosporiferis* hypophyllis, sparsis vel gregariis, subepidermalibus, erumpentibus, minutis; *uredosporis* globosis, subglobosis vel irregularis, prominenter echinulatis, $24 \times 16\,\mu$ ($20–28 \times 12–20\,\mu$); paraphysibus numerosis, clavatis, subhyalinis.

In vivis foliis *Brideliæ retusæ* Spr., Walayar, Malabar, 7–1–1948, T. S. Ramakrishnan et K. Ramakrishnan.

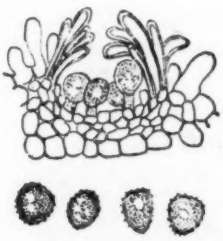


FIG. 7. *Uredo malabarica*—Section through uredium; urediospores (× 200)

The rusted leaves often present a mottled appearance when viewed from above owing to the large number of small spots. The sori are subepidermal. The club shaped paraphyses with subhyaline apices are more numerous towards the periphery of the sorus and are bent inwards. A peridium is not evident.

Schroeteriaster cingens (Syd.) Syd. (*Melampsora cingens* H. and P. Syd.; *Bubakia cingens* (Syd.) Mundk.) has been recorded on *Bridelia* spp. (Sydow and Butler, 1912; Mundkur, 1943). This rust has a peridium for the uredium and paraphyses are absent. Doidge (1924) has recorded *Uredo brideliæ* on *Fluggea microcarpa* from South Africa. The rust under study differs from *U. brideliæ* in possessing longer, more echinulate urediospores and the paraphyses form a fringe round the mouth of the sorus and not a palisade-like row as described in *U. brideliæ*. Hence this rust is considered to be a new species.

(49) *Uredo terminaliæ-paniculatæ* Ramakrishnan, T. S. and K. sp. nov.

Uredia hypophyllous, isolated or gregarious, each in the middle of a purple hypophyllous spot, subepidermal, with a distinct peridium; *urediospores* variable in shape, borne on very short, stout stalks, very light brown, echinulate 24 × 17 μ (18–32 × 12–20 μ).

On living leaves of *Terminalia paniculata* Roth., Walayar, Malabar, 7–1–1948, T. S. Ramakrishnan and K. Ramakrishnan.

Maculis hypophyllous, rubris; soris *uredosporiferis* hypophyllis, sparsis, vel aggregatis, subepidermalibus, peridio distincto; *uredosporis* globosis subglobosis vel ellipticis, echinulatis, sub brunneis, 24 × 17 μ (18–32 × 12–20 μ), pedicellis brevi, pinguibus.

In vivis foliis *Terminaliæ paniculatæ* Roth., Walayar, 7–1–1948, T. S. Ramakrishnan et K. Ramakrishnan.

Numerous isolated or coalescent purple spots develop on the leaves. The uredia are formed hypophyllously in the middle of the spots. They are subepidermal and covered by a multicellular peridium made up of thin walled cells. The peridium is thicker on the sides and consists of one layer of cells at the top. The urediospores are produced singly on very short stout pedicels.

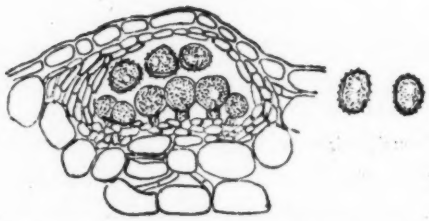


FIG. 8. *Uredo terminaliae paniculatae*—Section through uredium, urediospores (×200)

Uredo terminaliæ P. Henn., *U. buchenaviæ* Kern. and Whetzel. and *Aecidium terminaliæ* Ramakr. T. S. and K. have been recorded on allied host plants. This rust differs from the two species of *Uredo* in possessing a peridium.

(50) *Aecidium marsdeniæ* Ramakrishnan, T. S. and K. sp. nov.

Spots circular, amphigenous, pale yellow at first, later becoming dark brown, up to 15 mm. in diam.; *aecia* hypophyllous, rarely amphigenous, crowded in the spot, cupulate; margin of the peridium reflexed, white, lacerated cells of the peridium polygonal, unequally thickened, verrucose

24 × 16μ (18–32 × 11–22 μ), *aeciospores* subglobose to polygonal, orange yellow, 18× 12 μ (15–22 × 7–14 μ); epispore thin, smooth.

On living leaves of *Marsdenia volubilis* T. Cke., Burliar, Nilgiris, 22–7–1948, T. S. Ramakrishnan and K. Ramakrishnan.

Maculis amphigenis, orbicularibus, primo pallido flavo colore, deinde fusco-brunneo colore, usque 15 mm. diam.; *aeciis* hypophyllis, raro amphigenis, aggregatis, poculiformibus; pseudoperidii margine reflexo, albido, lacerato; cellulis polygoniis, inequaliter, incrassatis, verrucosis, 24 × 16 μ (18–32 × 11–12 μ); aeciosporis subglobosis vel polygonis, luteo colore, 18 × 12 μ (15–22 × 7–14 μ); episporio levi, tenui.

In vivis foliis *Marsdeniæ volubilis* T. Cke., Burliar, Nilgiris, 22–7–1948, T. S. Ramakrishnan et K. Ramakrishnan.

At first the spots are small about 5 mm. in diameter and yellowish in colour. Later these enlarge, become grayish brown, and the tissues of the leaf tear off and fall leaving holes. The aecia are clustered on the lower surface of the leaf in the spotted region. An occasional aecium is found on the upper surface also.

Puccinia marsdeniæ Diet. et Holw. has been recorded on *Marsdenia mexicana* (Saccardo, 1905). But no aecium has been described for this rust. The rust under study produces only the aecium and is therefore for the present included in the form genus *Aecidium*.

The writers are thankful to Dr. G. R. Bisby of the Commonwealth Mycological Institute, Kew, who kindly went through the manuscript and offered critical remarks. They also wish to thank the Superintendent, Royal Botanic Gardens, Calcutta, and the Government Lecturing and Systematic Botanist, Coimbatore for kindly identifying some of the host plants. Mr. M. Mohan Rao, of the Cinchona Department was kind enough to help us in the collection of the specimens of some of the host plants. We are indebted to Rev. Fr. J. Rapinat, S.J., of the Loyola College, Madras, for the Latin diagnoses.

References

Cummins, G. B. .. *Mycologia*, 1935, **27**, 605–07.

———— .. *Bull. Torr. Bot. Club*, 1945, **72**, 207.

Dietel, P. .. *Ann. Mycol.*, 1906, **4**, 421–23.

Doidge, E. M. .. *Bothalia*, 1926, **2**, 1–228.

Kern, F. D., and Whetzel, H. H. *Mycologia*, 1926, **18**, 41.

Mundkur, B. B. .. *Ibid.*, 1943, **35**, 544.

Mundkur, B. B., and Thirumalachar, M. J. *Ibid.*, 1945, **37**, 619–28.

Saccardo, P. A. .. *Syll. Fung.*, 1902, **16**, 318.

———— .. *Ibid.*, 1905, **17**, 334, 337.

———— .. *Ibid.*, 1912, **21**, 714–15.

Sydow, H. and P. .. *Monographia Uredinearum*, 1904, **1**, 334–35.

——— and Butler, E. J. .. *Ann. Mycol.*, 1912, **10**, 270.

Explanation of Plate VIII

(*a*) Urediospores of *Melampsora stereospermi*. ×300.

(*b*) Section through uredium of *M. stereospermi*. ×200.

(*c*) Section through telium of *M. stereospermi*. ×150.

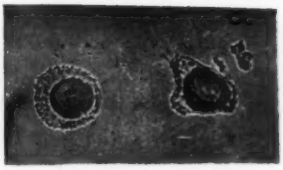
(*d*) Section through telium of *Chrysocelis indica*. ×300.

(*e*) *Cronartium fici* showing uredia and telia slightly enlarged.

(*f*) Section through a telium of *Goplana indica*. ×250.

(*g*) A cluster of teliospores of *G. indica*. ×300.

(*h*) Teliospore germination in *G. indica*. ×400.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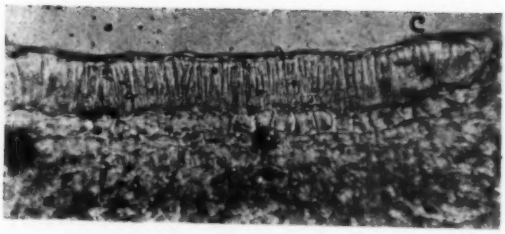
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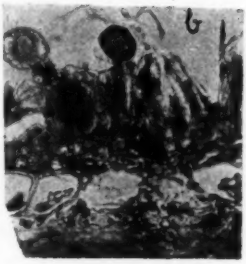
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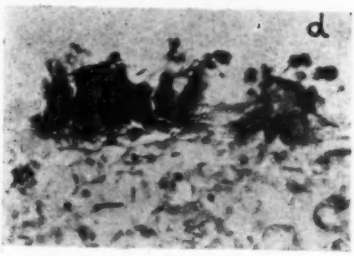
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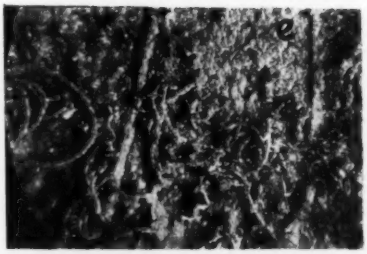
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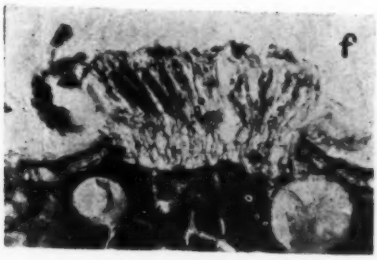
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THE NUTRITIVE AND VITAMIN VALUE OF GROUNDNUT

Part I. Vitamin B_1 Content of Groundnut *(Arachis hypogea)* as Affected by Variety

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(Department of Biochemistry, Indian Institute of Science, Bangalore)

Received April 2, 1948

The vitamin B_1 content of cereals and its variation as affected by variety has been the subject of study by several workers. Most of the investigations were carried out with cereals such as wheat, rice, maize, barley, etc.

There is no information available in literature on the influence of varietal differences on the vitamin B_1 content of groundnut (*Arachis hypogea*). The present investigation was, therefore, undertaken with a view to determining the variations that might exist in the vitamin B_1 content of a number of breeding stocks and trial varieties of groundnut. The results obtained in the present investigation are of considerable theoretical and practical importance in pointing out the importance of breeding for nutritional quality.

Experimental

The varieties of groundnut used in these experiments were raised under idcntical conditions on a light sandy loam with adequate fertilization in randomised blocks (plot size 13′ × 25′, consisting 12 rows placed 1′ apart). Preparatory cultivation was the same for all plots. The plots were manured with compost and superphosphate (2½ tons of compost and 100 lbs. of superphosphate per acre). Seeds were drilled. Sowing was done from 12th to 16th July 1946. The cultural practices which followed sowing were quite uniform for all the plots. The early varieties were harvested on 12th November 1946 and the late varieties on 16th December 1946. After harvest, the seeds were sun-dried till constant in weight, and stored in tins.

Preparation of the material for the estimation of vitamin B_1.—A representative sample of each variety was obtained and the pods were shelled. The oil was pressed out in hydraulic press and the pressed cake was then powdered. The powder was defatted with ether in Soxhlet in order to remove the residual oil. The defatted material was again powdered and passed through 60-mesh sieve. The sample thus prepared was used for the estimation of vitamin B_1. The estimations were carried out in duplicates and the average of the two values is given. The results are presented in Table I.

TABLE I

Influence of Variety on Vitamin B_1 Content of Groundnut

Description of material	Yield in lbs. per acre	Fat per cent.	Vitamin B_1 content			
			Micrograms per gm. of defatted flour	Micrograms per gram of seed	International units per gm. of defatted flour	International units per gm. of seed
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Spreading commercial varieties						
1. *Mauritius*—(G 0115) early, maturing in about 4½ months in Mysore. The growth is not vigorous, the pods are medium sized and mostly two seeded ..	452	43·2	10·1	4·4	3·4	1·5
2. *Mysore local*—(G 0120) is a very late, vigorous type, 5½–6 months duration, its branches often turn up near the tip. Pods are slender with a pronounced ribbed surface and a prominent beak and quite often 4–5 seeds are found in each pod. It is an edible variety ..	322	44·6	7·8	3·5	2·6	1·2
3. *Virginia*—(G 0284) is comparatively vigorous, takes about 10 days more than Mauritius for maturity. Pods are large, contain a greater number of 3 seeded pods ..	773	41·0	11·2	4·6	3·7	1·5
4. *Big Japan*—(G 0020) Quite vigorous same duration as Virginia. Some pods are extra large in size ..	490	42·6	13·4	5·7	4·5	1·9
5. *Carolina*—(G 0080) early, maturing in about 4½ months. The growth is not vigorous. The pods are two seeded ..	413	46·3	9·6	4·4	3·2	1·5
6. *Pondicherry*—(G 0126) Quite vigorous, takes about 10–15 days more to come to maturity than Carolina. Pods are larger and mostly 3 seeded	604	45·7	5·4	2·5	1·8	0·8
7. *Spreading, improved varieties*—D_3 (G 0788) a variety got from Punjab, parentage is not known, same characteristics as Big Japan ..	801	40·9	11·5	4·7	3·8	1·6
8. *Saloum*—(G 0963) external appearance and behaviour is same as Big Japan and is used as a control in the breeding plots ..		43·7	8·1	3·5	2·7	1·2
9. T_{28} (G 0997) a variety from U.P. ..	630	43·2	15·1	6·5	5·0	2·2
10. *A. H. 477*—(G 0771) Late variety, drought resistant. Pods large, rather smooth (Bassi selection) ..	882	46·5	10·3	4·8	3·4	1·6
11. *Pondicherry No. 8*—(G 0787) selection from (Bombay Department of Agriculture) Pondicherry variety ..	920	45·2	6·1	2·8	2·01	0·9

TABLE I—(*Contd.*)

Description of material	Yield in lbs. per acre	Fat per cent.	Vitamin B_1 content			
			Micrograms per gm. of defatted flour	Micrograms per gram of seed	International units per gm. of defatted flour	International units per gm. of seed
(1)	(2)	(3)	(4)	(5)	(6)	(7)
12. *Bassi*—(G 0019) similar to A.H. 477, late, large pods ..	462	46·3	10·8	5·0	3·6	1·7
13. *Philippine white*—(G 0125) seeds dirty white in colour, pods are larger and contain 3 seeds ..	490	43·1	10·5	4·5	3·5	1·5
14. *Arachis Nambyquaræ*—(G 0123) is a different species, not very vigorous and late in maturity (nearly 6 months) the pods are very large and the surface is more or less ribbed. The shell is thick and the seed coat is splashed red and white ..	294	43·3	10·8	4·7	3·6	1·6
Early-erect varieties (Commercial type).						
15. *Spanish*—(G 0168) is quite vigorous matures in 4 months, pods are small and two seeded, seed colour is flesh colour. On account of its early maturity, this type is exclusively grown in black cotton soils ..	..	45·7	11·2	5·1	3·7	1·7
16. *Valentia*—(G 0261) is very early, 3½ months duration, pods are medium, rather smooth, the beak is not pronounced. Seeds are dark red in colour ..	202	43·2	12·3	5·3	4·1	1·8
17. *Tennessee White*—(G 0226) Pods are very long and contain 4–5 seeds. Shell is thick. Seeds are flesh coloured	291	42·4	14·9	6·3	5·0	2·1
18. *Coreantis*—(G 0105) is very early. 3½ months, moderate in vigour and has large hairy leaves. Pods are medium, seeds are deep red in colour ..	363	47·2	7·8	3·7	2·6	1·2
19. *Small Japan*—(G 0131) quite vigorous, matures in 4 months, pods are small and two seeded. Seeds are red ..	372	45·1	10·9	4·9	3·6	1·6
20. *African White*—(G 0003) duration same as Tennessee White, seeds flesh coloured ..	360	47·8	8·9	4·3	3·0	1·4
21. *Spanish*—(G 0786) from Bombay ..	..	45·3	9·1	4·1	3·0	1·4
22. *African Red*—(G 0001) (Commercial type-Africa) It is under extensive cultivation in Africa. Pods are bold, two seeded. Seeds are reddish brown in colour ..	870	44·6	13·6	6·1	4·5	2·0
23. *A. K. 8-11*—(G 0784) Central Provinces, similar to Bunch Virginia. Pods are still larger and 3 seeded pods are quite common. Under dry conditions in Mysore, pods are frequently arrested in development	452	45·7	11·9	5·4	4·0	1·8

TABLE I—(*Contd.*)

Description of material	Yield in lbs. per acre	Fat per cent.	Vitamin B_1 content			
			Micrograms per gm. of defatted flour	Micrograms per gram of seed	International units per gm. of defatted flour	International units per gm. of seed
(1)	(2)	(3)	(4)	(5)	(6)	(7)
24. *H. G_1*—(G 0106) is a standard improved variety in Mysore, duration 4½ months, not vigorous but drought resistant. Pods are medium-sized with 2–3 seeds and flesh coloured. ..	870	44·6	14·1	6·3	4·7	2·1
25. *Bunch Virginia*—(G 0291) is about a week later in maturity than H. G_1 pods are bold, 2 seeded. Seeds are flesh coloured. (In U.S.A. it is a commercial type) .	583	47·2	7·7	3·6	2·6	1·2
26. *Castlecary* (G 0970) an improved variety from Africa where it is under extensive cultivation ..	871	46·8	12·5	5·9	4·2	2·0
27. *Samaru 32*—(G 0973) an improved variety from Africa ..	884	49·7	13·9	6·9	4·6	2·3
28. *Satara grey*—(G 0791) an improved variety from Bombay ..	990	45·3	13·7	6·2	4·6	2·1
Hybrids						
29. *(G 0553)*—Spanish × Small Japan (erect, early) still in breeding plots	690	47·2	17·2	8·1	5·7	2·7
30. *(G 0460)*—Spanish × Big Japan (erect, early) still in breeding plots	660	50·2	15·5	7·8	5·2	2·6
31. *(G 0385)*—Spanish × Rhodesia (erect, early) still in breeding plots	540	46·3	12·9	6·0	4·3	2·0
32. *(G 0341)*—Tennessee White × Valentia (erect, early bold) still in breeding plots ..	690	44·2	8·8	3·9	2·9	1·3
33. *(G 0549)*—Tennessee White × H.G_1 erect, early, bold) still in breeding plots ..	645	48·5	15·9	7·7	5·3	2·6
34. *(G 0184)*—Spanish × Carolina (spreading late) is quite vigorous, and pods are 3 seeded	1080	43·4	13·2	5·7	4·4	1·9
35. *(G 0779)*—Small Japan × Mysore local (very late, resembles Mysore local) ..	535	44·1	14·5	6·4	4·8	2·1
36. *(G 0717)*—Small Japan × Transvaal (erect, early, seeds are red) ..	390	44·5	11·5	5·1	3·8	1·7
37. *(G 0173)*—Spanish × Barbadous H. G_2 (erect, early pods are quite large and 3 seeded, heavier yielder than Spanish) ..	625	48·3	16·2	7·5	5·4	2·5
38. *(G 0343)*—Valentia × H. G_1, erect, early, pods are thin and quite long often containing 4–5 seeds, seeds are red in colour). This is considered to be edible type ..	1065	44·2	8·7	3·8	2·9	1·3

Thiamine was determined by the chemical method of Harris and Wang (1941) as modified by Reddi and Giri (1946).

Discussion

It is clear from the above results that the differences in vitamin B_1 content of various samples of groundnut are of considerable magnitude. No correlation between the average yields and vitamin B_1 content of various varieties investigated was observed. This shows definitely that vitamin content should not be overlooked in breeding better varieties. In the past too much emphasis has been placed on such factors as yield, disease resistance, flavour, draught resistance, etc., and very little attention was paid to the nutritive value. According to Maynard (1943) genetic and climatic factors have very great effect on the vitamin content of plants.

In addition to variety which affects the vitamin content, soil type, fertilization, locality, climate, season and stage of maturity when harvested may also affect the vitamin content.

Johannson and Rich (1942) and Hoffer, *et al.* (1944) found no relationship between the vitamin B_1 content of Canadian hard red spring wheat and the type of soil on which it was grown.

The influence of different types of manures on the vitamin B_1 content of cereals and groundnut is fully discussed in Part V of this series (Reddi and K. V. Giri).

Several workers (Hunt, 1927; Murri, 1939; Booth, 1940; Slater and Rial, 1941; Downs and Cathcart, 1941; Shultz, *et al.*, 1941; Conner and Straub, 1941; Whiteside and Jackson, 1943; and Hoffer and Geddes, 1944) observed variations in the vitamin B_1 content of wheat which were attributed to differences in climate and locality. Whiteside and Jackson (1943); Hoffer, *et al.* (1944) have also observed significant seasonal variations in vitamin B_1 content of Canadian wheat.

Variations in the vitamin B_1 content which were attributed to differences in stage of maturity when harvested were observed by several workers (Murri, 1939; Burkholder and McVeigh, 1942; Geddes and Levine, 1942).

It is clear from the above observations that the varietal and to some extent soil and climatic factors influence the vitamin content. In view of the recent developments indicating the importance of vitamin B_1 in nutrition it is desirable that more attention should be paid to produce crops of highest nutritional quality for man and animals. In order to achieve this object the plant-breeder should have the nutritional value of crop as one of the criteria for evolving new varieties.

Summary

1. Thirty-eight varieties of groundnut (*Arachis hypogea*) grown under identical conditions at Mysore Government Agricultural Farm, Hebbal, were analysed for their vitamin B_1 content.

2. Wide differences were observed in vitamin B_1 content of various varieties, the value ranging from 5·4 to 17·2 micrograms per gram of defatted flour.

3. No correlation between the average yields of various varieties and their vitamin B_1 content was observed.

4. The differences in vitamin B_1 content are attributable mainly to variations in variety, as all the other conditions under which they were grown are identical.

5. Other factors which influence the vitamin B_1 content and the possible significance of the results obtained in the present investigation in breeding programmes are discussed briefly.

Acknowledgments

Our thanks are due to Dr. L. S. Doraiswamy and his assistants for the supply of the material grown on their breeding plots in the Hebbal Farm, the Department of Agriculture, Government of Mysore.

References

Booth, R. G. .. *J. Soc. Chem. Ind.*, 1940, **59**, 181.

Burkholder, P. R. and McVeigh, I. .. *Proc. Nat. Aca. Sci.*, 1942, **28**, 440.

Conner, R. T. and Straub, G. J. .. *Cereal Chem.*, 1941, **18**, 671.

Downs, D. E. and Cathcart, W. H. .. *Ibid.*, 1941, **18**, 796.

Geddes, W. F. and Levine, M. N. .. *Ibid.*, 1942, **19**, 547.

Harris, L. J. and Wang, Y. L. .. *Biochem., J.*, 1941, **35**, 1050.

Hoffer, A., Alcock, A. W. and Geddes, W. F. .. *Cereal Chem.*, 1944, **21**, 210.

Hunt, C. H. .. *Ohio Agric. Exp. Sta. Bn.* No. 415, 1927.

Johannson, H. and Rich, C. E. .. *Cereal Chem.*, 1942, **19**, 388.

Maynard, L. A. .. *Chem. & Eng. News*, 1943, **21**, 217.

——— and Beeson, K. C. .. *Nutrit. Abst. Revs.*, 1943, **13**, 155.

Murri, I. K. .. *Ibid.*, 1939, **10**, 3407.

Reddi, K. K. and Giri, K. V. .. *Science and Culture*, 1946, **11**, 507.

——— .. *Proc. Ind. Acad. Sci.* (in press).

Shultz, A. S., Atkin, L. and Frey, C. N. *Cereal Chem.*, 1941, **18**, 106.

Slater, E. C. and Rial, E. J. .. *Austral. Chem. Inst. J. & Proc.*, 1941, **8**, 71.

Whiteside, A. G. O. and Jackson, S. H. *Ibid.*, 1943, **8**, 71.

——— .. *Cereal Chem.*, 1943, **20**, 542.

THE NUTRITIVE AND VITAMIN VALUE OF GROUNDNUT

Part II. The Presence of Free and Combined Forms of Vitamin B_1 in Groundnut (*Arachis hypogea*)

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Vitamin B_1 occurs in plants and animals in both free and combined forms. In combined form it is found to occur mostly as cocarboxylase, a coenzyme, which was first noticed in yeast by Auhagen (1933) working in Euler's laboratory. Besides yeast it occurs in liver, kidney, heart and blood. It is also present in muscle in small amounts (Ochoa and Peters, 1938; Westernbrink and Jansen, 1938).

The cocarboxylase was isolated in crystalline form from yeast by Lohmann and Schuster (1937), who found that it was composed of one molecule of thiamine and two molecules of phosphoric acid combined as the pyrophosphate. Since then extensive investigations were carried out on the physiological significance and chemical characteristics of the coenzyme. Cocarboxylase has been synthesised by enzymatic and chemical methods (Kinnersley and Peters, 1937; Euler and Vestin, 1937; Tauber, 1937; Ochoa and Peters, 1938; Stern and Hoffer, 1937; Weijlard and Tauber, 1938). The synthetic Cocarboxylase is found to behave in every respect similar to cocarboxylase prepared from yeast.

According to Lohmann and Schuster (1937) most of the thiamine in yeast is in the form of pyrophosphate but in rice polish it occurs largely in free form. In animal tissues, the thiamine is stated to be entirely in phosphorylated form (Lipman, 1937).

There is, however, very little information available in literature regarding the occurrence of cocarboxylase in plant tissues. Tauber (1937) investigated a series of plants for cocarboxylase and compared the results with their vitamin B_1 content and observed that only a very small portion of the vitamin B_1 is present in the plants in the form of its phosphoric ester.

The existence of vitamin B_1 combined with substances other than phosphoric acid was reported by some workers. Evidence has been adduced to show that it may unite with other substances when mixed with bile (Melnick, Robinson and Field, 1941). In milk approximately half of vitamin B_1 is found to be present combined with protein (Houston and Kon, 1939; Holiday and Harry, 1941).

The object of the present study is to investigate the occurrence of free and combined forms of vitamin B_1 in different varieties of groundnut and the nature of the combination. The varieties of groundnut used in this investigation were obtained from the Mysore Agricultural Farm, Hebbal. They were grown under identical conditions.

Experimental

Preparation of the material.—The material for analysis was prepared in the same way as described before in Part I of the series.

Methods employed

(1) *Total vitamin* B_1 was estimated according to the method given in Part I of the series.

(2) *Free vitamin* B_1 (Harris and Wang, 1941).—One gram of material was taken in a conical flask and 15 c.c. of 0·02 N HCl were added and mixed well. To this 5 c.c. of sodium acetate-acetic acid buffer pH 4·0 were added. The flask was immersed in a boiling water-bath for 5 minutes (to inactivate phosphatase) and cooled to room temperature. The volume was made up to 25 c.c. and vitamin B_1 was determined. The value thus obtained is the amount of free vitamin B_1.

(3) *Bound vitamin* B_1.—The difference between the values obtained for " free vitamin B_1" and " total vitamin B_1", after digestion with papain, gives the value for " bound vitamin B_1".

The results of the analyses are presented in Table I.

Table I

Free and Bound Forms of vitamin B_1 *in groundnut* (Arachis hypogea)

Description of material	Vitamin B_1 content				
	Total vitamin B_1 (micrograms per gram of defatted flour)	'Free' vitamin B_1 (micrograms per gram of defatted flour)	Per cent. of free vitamin B_1	"Bound" vitamin B_1 (micrograms per gram of defatted flour)	Per cent. of "Bound" vitamin B_1
H. G_1 (G 0106) ..	14·0	7·1	50·7	6·9	49·3
T 25 (G 0997) ..	15·1	8·3	54·9	6·8	45·1
Hybrid (G 0779) ..	14·5	7·4	51·0	7·1	49·0
Arachis Nambyquaræ (G 0123)	10·8	5·5	50·9	5·3	49·1
African white (G 0003) ..	8·9	5·1	57·3	3·8	42·7
Hybrid (G 0173) ..	16·2	8·9	54·9	7·3	45·1
Bunch Verginia (G 0291) ..	7·7	4·3	55·8	3·4	44·2
African red (G 0001) ..	13·6	6·7	49·3	6·9	50·7
Hybrid (G 0553) ..	17·2	9·2	53·5	8·0	46·5
Hybrid (G 0184) ..	13·2	6·8	51·5	6·4	48·5

From the above results, it is clear that nearly 50 per cent. of total vitamin B_1 is in the " free form " and the rest is in the " bound form ". It is, therefore, of interest to find the nature of the carrier of the bound form of vitamin B_1 in groundnut.

The nature of the carrier of the bound vitamin B_1

In order to determine the nature of the carrier of the " bound form ", the following experiments were carried out. The variety HG_1 was used for these experiments.

(1) *Digestion with papain.*—One gram of defatted flour of the sample was taken in a conical flask and 15 c.c. of 0·02 N HCl were added and mixed well. To this mixture 5 c.c. of sodium acetate-acetic acid buffer of pH 5·0 were added and digested at temperature of 60° C. for one hour with 0·1 gm. of papain with frequent shaking. At the end of the period, the volume was made up to 25 c.c. and centrifuged. The vitamin B_1 present in the clear centrifugate was estimated. The results are given in Table II.

(2) *Digestion with papain and takadiastase.*—One gram of defatted flour of the sample was taken in a conical flask and 15 c.c. of 0·02 N HCl were added and mixed well and kept in boiling water-bath for 10 minutes with constant stirring. Then 5 c.c. of sodium acetate and acetic acid buffer pH 4·0 were added and mixed well and allowed to cool. To this 0·1 gm. of papain (prepared from fresh latex of papaya) and 0·1 gm. of takadiastase (Parke Davis & Co.) were added. The contents were mixed well and incubated overnight at 40° C. with the addition of a few drops of toluene as preservative. At the end of the period, the volume was made up to 25 c.c. centrifuged and vitamin B_1 present in the clear centrifugate was estimated. The results are given in Table II.

(3) *Extraction with 10 per cent. NaCl.*—5 gms. of defatted flour were weighed into a stoppered bottle and 40 c.c. of 10 per cent. NaCl were added and the mixture was shaken in a mechanical shaker for one hour to ensure thorough mixing and kept overnight in the refrigerator. At the end of this period, this was centrifuged. The clear centrifugate was taken for the following experiments.

Extract

(*a*) An aliquot of the extract corresponding to 1 gram of defatted flour was taken and the pH was adjusted to 5·0 and vitamin B_1 was estimated without digestion with any enzyme. The results are given in Table II.

(*b*) An aliquot of the extract corresponding to 1 gram of defatted flour was taken in a conical flask and the pH was adjusted to 5·0 and digested

with 0·1 gram of papain at 60° C. for one hour and vitamin B_1 was estimated after digestion. The results are given in Table II.

Precipitation with Trichloro-acetic acid.—3 gms. of powder were weighed into a stoppered bottle and 30 c.c. of 4 per cent. trichloro-acetic acid were added and the mixture was shaken in a mechanical shaker for one hour to ensure thorough mixing and kept overnight in the refrigerator. At the end of the period it was centrifuged. The extract is free from protein. 10 c.c. of centrifugate corresponding to one gram of powder were taken for estimation of vitamin B_1. The vitamin B_1 was estimated without digesting with any enzyme. The results are given in Table II.

TABLE II

Vitamin B_1 content of Groundnut (defatted flour) as determined by Various Methods of Extraction

Procedure employed	Vitamin B_1 content micrograms per gram of defatted flour	Percentage of total vitamin B_1 extracted*
1. Extraction with 0·02N HCl ..	7·2	51·1
2. Digestion with papain ..	14·0	100
3. Digestion with papain and takadiastase ..	14·1	100
4. 10 per cent. NaCl extract ·		
(a) Without papain digestion ..	6·9	48·9
(b) With papain digestion ..	13·8	97·9
5. 4 per cent. trichloro-acetic acid extract ..	7·1	50·4

* The vitamin B_1 content obtained after digestion with papain and takadiastase is the total vitamin B_1.

DISCUSSION

The foregoing results show that in groundnut at least 50 per cent. of vitamin B_1 exists in bound form, which cannot be extracted with water and 0·02 N HCl. Digestion with papain, however, completely transforms the " bound " vitamin into free vitamin. The values obtained for total vitamin B_1 after digestion with papain or with papain and takadiastase are the same, thereby showing that takadiastase which contains phosphatase has no effect on bound vitamin B_1. The results thus indicate that the bound vitamin B_1 is not cocarboxylase, but it exists bound to protein, which on digestion with papain is transformed into free vitamin. Further, the bound form of the vitamin can be extracted with 10 per cent. NaCl and precipitated with 4 per cent. trichloro-acetic acid. The existence of such a large percentage of vitamin B_1 in bound form shows that the bound vitamin has some physiological function which may be different from the one exerted by free vitamin B_1. It is also probable that the bound vitamin B_1 may function as an important catalytic system in plants.

In addition to vitamin B_1, other vitamins are known to exist bound to protein. Thus, Kuhn and Wendt (1938) postulated that pyridoxine occurs in non-dialysable form, presumably combined with protein. Niemeijer (1946) reported two forms of nicotinic acid in blood (free and bound forms). According to him the bound form is in combination with protein.

Summary

1. Ten varieties of groundnut were analysed for free and bound forms of vitamin B_1. About 50 per cent. of total vitamin exists in the bound form.

2. Digestion with papain or a mixture of papain and takadiastase have the same effect in transforming the bound vitamin into free form. The bound vitamin B_1 is not a cocarboxylase.

3. The bound form of the vitamin can be extracted completely with 10 per cent. NaCl and can also be precipitated with 4 per cent. trichloroacetic acid. It, therefore, exists bound to protein.

References

Auhagen, E. .. *Ztschr. Physiol. Chem.*, 1933, **204**, 149.
——— .. *Biochem. Ztschr.*, 1933, **258**, 330.
Euler, H. and Vestin, R. .. *Naturwiss.*, 1937, **25**, 416.
Holiday, Nellie and Harry, J. Deael, Jr. .. *J. Biol. Chem.*, 1941, **140**, 555.
Houston, J. and Kon, S. K. .. *Nature*, 1939, **143**, 558.
Kinnersley, H. W. and Peters, R. A. .. *J. Soc. Chem. Ind.*, 1937, **56**, 934.
Kuhn, R. and Wendt, G. .. *Physiol. Chem.*, 1938, **256**, 127.
Lipman, F. .. *Enzymologia*, 1937, **4**, 65.
Lohmann, K. .. *Anges. Chem.*, 1937, **50**, 221.
——— and Schuster, P. .. *Naturwiss*, 1937, **25**. 26.
——— .. *Biochem. Ztschr.*, 1937, **294**, 188.
Melnick, D. Robinson, W. D. and Field, H. Jr. .. *J. Biol. Chem.*, 1941, **138**, 49.
Neimeijer, J. A. .. *Rec. Trav. Chim.*, 1946, **65**, 84.
Ohoa, S. and Peters, R. A. .. *Biochem. J.*, 1938, **32**, 1501.
Simola, P. .. *Biochem. Ztschr.*, 1932, **254**, 229.
Stern, K. and Hoffer, V. .. *Science*, 1937, **85**, 483.
——— .. *Enzymologia*, 1937, **3**, 82.
Tauber, H. .. *Science*, 1937, **86**, 180.
——— .. *J. Amer. Chem. Soc.*, 1937, **60**, 2263.
——— .. *Proc. Soc. Expt. Biol. & Med.*, 1937, **37**, 541.
——— .. *Enzymologia*, 1937, **2**, 171.
Weijlard, J. and Tauber, H. .. *J. Amer. Chem. Soc.*, 1938, **60**, 2263.
Westenbrink, H. C. K. and Jansen, B. C. P. *Acta. Brevia, neerl.*, 1938, **8**, 119.

THE NUTRITIVE AND VITAMIN VALUE OF GROUNDNUT

Part III. Physiological Availability of Vitamin B_1 in Groundnut (*Arachis hypogea*)

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The physiological availability of Vitamin B_1 in foodstuffs particularly in yeast has been investigated by several workers. Yeast is known to be a good source of Vitamin B complex and a considerable amount of Vitamin B_1 exists in phosphorylated form (Lohmann and Schuster, 1937). However, studies by Walker and Nelson (1933) showed that Vitamin B_1 in fresh yeast is not readily available for absorption in gastro-intestinal tract of the rat. This may be attributable to the resistance of the live yeast cells to digestion.

Parsons and his associates (1942) at the University of Wisconsin presented evidence to show that the water-soluble vitamins in fresh yeast are not available to man.

Melvin, Hochberg, Daniel, Melnick and Bernard (1945) investigated the availability of Vitamin B_1 in yeast by means of human bio-assay technique described by Melvin, Hochberg and Oser (1945). According to them, the physiologically available thiamine in the live yeast is calculated to be only 17 per cent.

In Part II of this series it was shown that there exist in groundnut two forms of Vitamin B_1—one is free form and the other is bound to protein. The object of the present study is to find out the extent of the availability of Vitamin B_1 present in groundnut by rat growth technique.

Experimental

Young albino rats were used for these experiments. The growth-promoting property of Vitamin B_1 is applied as the criterion of the availability.

Selection of the diet.—An examination of literature shows that there is no uniformity in the character of the basal rations for Vitamin B_1 studies. Some workers used starch exclusively as source of energy, some used starch and fat, while others employed sucrose in their dietary mixtures. It was found by Herbert and Lepkovsky (1929) that the growth obtained in the

absence of Vitamin B_1 depends largely upon the basal diet used. According to them the following factors are important; (1) the amount of protein, (2) the kind and amount of carbohydrate used, (3) the amount of fat. The last named has special importance (Evans and Lepkovsky, 1928). Evans, *et al.* (*loc. cit.*) studied about seven diets containing varying amounts of sucrose, proteins and fat. Of all the diets tested, the most sensitive for growth studies was found to be a sucrose-protein diet of the following composition.

Composition of the basal diet used

Casein (free from Vitamin B_1) ..	20
Sucrose	70
Salt mixture	4
Autoclaved yeast	10

This diet was supplemented with two drops of cod-liver oil daily.

Preparation of casein free from Vitamin B_1.—The casein was prepared according to the method of Evans and Lepkovsky (1928).

Autoclaved yeast.—Whole dried yeast was spread in pans to a depth of less than one inch and autoclaved for 5 hours at 18–20 pounds pressure. This yeast was used as a source of thermostable water-soluble factor throughout the experiment.

Salt mixture (McCollum).—Sodium chloride 51·0; crystals of magnesium sulphate, 159·6; monobasic sodium phosphate 104·1; monobasic calcium phosphate, 162·0; dibasic potassium phosphate, 286·2; ferric citrate, 35·4 and calcium lactate, 390·0.

Preparation of test substance.—Groundnut flour was prepared in the same way as described before in Part I of the series.

Different forms of Vitamin B_1 in groundnut flour

Total Vitamin B_1 ..	14·0	microgram/gm.	of defatted flour.
" Free form "	7·1	"	"
" Bound form "	6·9	"	"

Procedure.—Healthy albino rats 24–27 days old weighing from 45-54 grams and of known nutritional history were used as experimental animals. The young animals were taken from stock colony and placed in individual cages with raised screen floors to prevent access to excreta. The rats were divided into three groups, each group containing 6 rats (three males and three females).

The basal diet described above was given (12 grams per rat). The storage capacity for Vitamin B_1 is so low that it is not necessary to deplete

the animals preliminary to feeding (Evans and Lepkovsky, 1928). However, they were fed on basal diet for five days to be on the safe side. The groundnut flour was fed at a level providing 5 micrograms of thiamine per rat per day which approximates the growth requirement (Williams and Spies, 1938) and was compared with a control group which were fed 5 micrograms of pure thiamine hydrochloride per day. Thiamine in solution was delivered daily through pipette into mouth. The observations of body weight were made once in five days. In the complete absence of Vitamin B_1, an initial gain in weight for the first week was noticed and this was followed by a decline and food intake has also fallen down. There was death of one rat on 39th day. The other two groups showed more or less identical growth over a period of 40 days. This indicates that thiamine in groundnut is completely available to the rats as the crystalline thiamine.

The results are presented in Table I and Fig. 1.

TABLE I

Availability to rats of thiamine in groundnut

Groups		Body weight of rats (gm.)								
		1st day	5th	10th	15th	20th	25th	30th	35th	40th
Groundnut flour corresponding to 5 micrograms of Vitamin B_1 per day per rat (A)	1..	45	49	58	67	77	82	86	94	101
	2..	49	56	65	69	82	90	93	99	105
	3..	47	51	54	68	76	86	91	98	108
	4..	52	59	70	83	92	99	106	114	118
	5..	48	54	65	79	88	98	99	103	110
	6..	53	56	63	74	87	98	102	109	114
	Average ..	49·0	54·2	62·5	73·3	83·7	92·1	96·1	102·7	109·3
Thiamine hydrochloride 5 micrograms per day per rat (B)	1..	54	63	75	83	88	100	105	110	116
	2..	49	54	57	70	82	95	99	100	111
	3..	53	55	61	69	78	83	94	102	105
	4..	49	56	60	63	73	75	83	87	89
	5..	46	49	54	68	79	87	91	99	104
	6..	51	57	58	66	74	88	94	105	110
	Average ..	50·8	55·7	60·8	69·8	80·7	88·1	94·3	105·5	105·8
Control (C)	1..	45	57	59	59	57	52	49	42	39
	2..	46	52	56	57	51	48	47	42	40
	3..	52	62	65	66	64	58	53	49	43
	4..	54	55	54	54	51	46	40	39	35
	5..	48	53	53	52	48	45	42	35	death
	6..	45	50	51	49	48	43	42	37	34
	Average ..	48·0	54·8	56·3	56·1	53·5	47·0	45·5	40·0	38·2

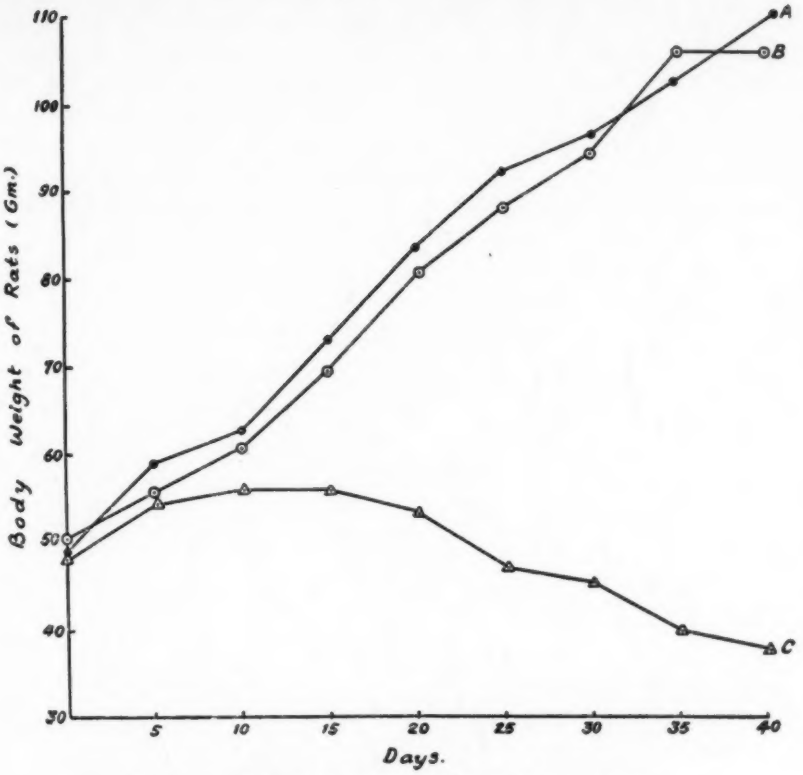

FIG. 1. Availability to Rats of Thiamne in Groundnut

A = Groundnut flour corresponding to 5 micro grams of vitamin B_1 per rat

B = Thiamine hydrochloride 5 micrograms per day per rat

C = Control

DISCUSSION

The results show that the Vitamin B_1 present in groundnut is completely available just as pure thiamine. Although part of the vitamin in groundnut is present in bound form, the absorption of the vitamin is not affected. In view of the reported findings by several workers on the low availability of Vitamin B_1 in yeast, it is desirable that the physiological availability of Vitamin B_1 in foodstuffs should be studied. The results reported in the present investigation are therefore of considerable importance in indicating the complete availability of the vitamin present in groundnut which as reported earlier is a rich source of Vitamin B_1.

SUMMARY

The physiological availability of Vitamin B_1 in groundnut was studied by rat growth technique. The results indicate that Vitamin B_1 in groundnut is completely available for growth.

REFERENCES

Evans, M., Herbert and Samuel Lepkovsky .. *Science*, 1928, **28**, 298.
——— .. *J. Nutrition*, 1929, **2**, 1.
Lohmann, K. and Schuster, P. .. *Naturwiss*, 1937, **25**, 26.
Melvin, Hochberg, Daniel Melnick and Bernard, L. Oser .. *Ibid.*, 1945, **30**, 201.
Melvin, Hochberg and Bernard, L. Oser .. *Ibid.*, 1945, **30**, 67.
Parsons, H. T., Williamson and Johnson, M. L. .. *Ibid.*, 1945, **29**, 373.
———, Collard, J., Gardner, J., Storey, F. M. and Peterson, W. H. *Federation Proc.*, 1945, **1**, 129.
——— and Collard, J. .. *J. Amer. Deit. Assn.*, 1942 *a*, **18**, 805.
Walker, R., and Nelson, E. M. .. *Amer. J. Physiol.*, 1933, **103**, 25.
Williams, R. R. and Spies, T. D. .. *Vitamin B_1 in Medicine*, 1938, p. 313 (Macmillan & Co.).

THE NUTRITIVE AND VITAMIN VALUE OF GROUNDNUT

Part IV. Influence of Germination on the Vitamin B_1 Content of Groundnut (*Arachis hypogea*)

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In recent years the changes in vitamin B_1 content of seeds during germination have been studied by several workers. Some of them reported decrease in vitamin B_1 content during germination of seeds (Hlavaty, 1929; Kucera, 1929; Kutty, 1937; Rose and Phipard, 1937). Other workers (Burkholder and McVeigh, 1942, 1945; Burkholder, 1943 and Vernon and Robert, 1943) have, however, shown that in the case of seeds with high protein content thiamine remains unchanged during germination.

The present investigation was undertaken with a view to throw more light on the transformation of vitamin B_1 during germination of groundnut. Groundnut being a very rich source of vitamin B_1, is best suited for such studies. Further, in view of our observations on the existence of bound form of vitamin B_1 in groundnut it was of interest to investigate the changes in the bound form of vitamin B_1 during germination. The changes brought about in the " bound and free " forms of vitamin B_1 during germination of seeds have not been studied before and the results obtained in the present study are of interest in throwing light on the mechanism of transformation of various forms of vitamin B_1 during germination of seeds. In this paper, the results of these investigations are presented and discussed.

Experimental

The material for these studies was obtained from Mysore Government Agricultural Farm, Hebbal.

Germination.—The seeds were germinated on moistened filter-papers in large Petri dishes, kept in a darkened room. The seeds were analysed for vitamin B_1 content after varying periods of germination.

Preparation of the material for vitamin B_1 analysis.—Weighed amounts of germinated seeds were cut into small bits and spread on a filter paper and dried over anhydrous calcium chloride in a vacuum desiccator for 24 hours to free it from moisture. The samples were powdered and defatted.

The " free and bound " forms of vitamin B_1 were determined according to methods described before in Part II of the series.

The results are presented in Table I and Fig. 1. The results are expressed as micrograms of vitamin B_1 per gram of defatted flour.

TABLE I

Changes in Vitamin B_1 Content of Groundnut during Germination

Period of germination (hours)	" Free form " micrograms per gram of defatted flour	" Bound form " micrograms per gram of defatted flour	Total Vit. B_1 micrograms per gram of defatted flour
0	7·2	6·9	14·1
24	9·0	1·5	10·5
48	7·9	nil	7·9
72	6·3	do	6·3
96	5·7	do	5·7
120	4·6	do	4·6
144	3·9	do	3·9

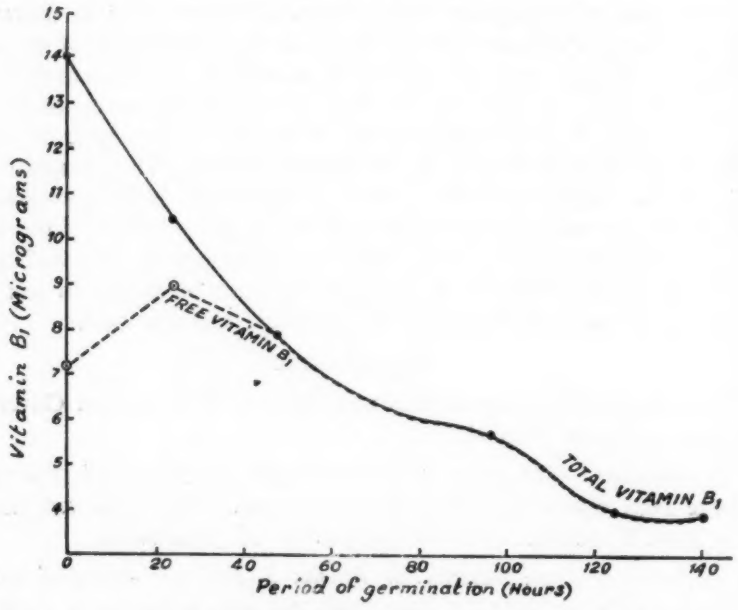

FIG. 1. Changes in Vitamin B_1 Content of Groundnut during Germination

● ——— ● Total Vitamin B_1

⊙ - - - ⊙ Free Vitamin B_1

Discussion

From the data in the above table, it is clear that there are two significant changes occurring during germination; (1) decrease in total vitamin B_1 content, and (2) release of " bound form " of vitamin B_1 into " free form".

The decrease in vitamin B_1 content of groundnut during germination is of considerable physiological significance. In recent years several workers have adduced evidence to show that vitamin B_1 is very essential for the growing embryo. During the initial stages of development, the embryo being heterotropic, depends mostly on the reserve material contained in the cotyledons. The idea that thiamine is quite indispensable during germination was first initiated by Kogl and Haagen Smith (1936) and later elaborated by Bonner (1937) and Robbins and Bartley (1937). Thiamine present in the seed is being utilised by the embryo in the early stages of germination of the seed as it cannot synthesise the vitamin at this stage. Hlavaty (1929) has shown that in germinating legume seeds synthesis does not begin till about a month later and then only if the plants are kept in the sun. Similar observations were recorded by Bonner and Greene (1938), Rytz Jr. (1939). Although it is now well established that thiamine is quite essential for the growing embryo which utilises the vitamin present in the seed during germination, very little is known regarding the mechanism by which the vitamin is augmented in plant tissues undergoing renewed growth activity. The manner in which the thiamine is destroyed in the seed during germination is not known. In the light of the observations of Bonner and Buchman (1938) that in phycomyces, the thiazole component is destroyed after it has been absorbed by a mature culture, the rapid decrease in vitamin B_1 content during germination suggests that probably similar type of destruction is taking place in the seed.

Another change during germination observed in the present investigation is the transformation of the vitamin from " bound form " into " free form ". It was shown already in Part II of the series that in groundnut, the " bound form " is protein-B_1 complex. The " bound form " is rendered free during germination. It is well known that the protein reserves of seeds are subjected to hydrolysis during germination by proteases, contained in the seed. These enzymes increase in activity in the seed during germination and as a result of the hydrolytic action of the proteolytic enzymes, the proteins are rendered soluble thereby releasing the " bound vitamin B_1" into " free form ".

SUMMARY

1. The changes in vitamin B_1 content of groundnut during germination were studied.

2. There is decrease in total vitamin B_1 content as the germination advances.

3. The " bound form " of vitamin B_1 is rendered free during germination.

4. The probable causes for the decrease in vitamin B_1 and the mechanism of transformation of " bound vitamin B_1" into free form during germination are discussed.

REFERENCES

Bonner, J. .. *Science*, 1937, **85**, 184.

——— and Buchman, E. R. .. *Proc. Nat. Acad. Sci.* (Washington), 1938, **24**, 431.

——— and Greene, J. .. *Bot. Gaz.*, 1938, **100**, 226.

Burkholder, P. R. .. *Science*, 1943, **97**, 562.

——— and McVeigh, I. .. *Proc. Nat. Acad. Sci.* W. S., 1942, **28**, 440.

——————— .. *J. Plant. Physiol.*, 1945, **20**, 301.

Chick, H. and Delf, E. M. .. *Biochem. J.*, 1919, **13**, 199.

Hlavaty, J. .. *Compt. rend. Soc. Biol.*, 1929, **100**, 587.

Kogl, F. and Haagen, Smith, A. J. .. *Zeit. Physiol. Chem.*, 1936, **243**, 209.

Kucera, C. .. *Z. Tierziicht*, 1929, **13**, 387 (*Amer. Chem. Abs.*, 1930, **24**, 2491).

Kutty, S. V. .. *Congr. Inter. Tech. Chim. Ind. Agr. Compt. rend. Congr.*, 1937, **1**, 119.

Robbins, W. J. and Bartley, M. A. .. *Science*, 1937, **85**, 246.

Rose, N. S. and Phipard, E. M. F. .. *J. Nutrition*, 1937, **14**, 55.

Rytz, W. (Jr.) .. *Ber. Schweizbot. Ges.*, 1939, **49**, 339.

Vernon, H. C. and Robert, L. L. .. *Proc. Soc. Exptl. Biol. Med.*, 1943, **54**, 53.

THE NUTRITIVE AND VITAMIN VALUE OF GROUNDNUT

Part V. The Influence of Manuring on Vitamin B_1 Content of Groundnut (*Arachis hypogea*)

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The influence of organic manures on the nutritive value of cereals has been the subject of spirited controversy among the various workers in the field during the past two decades.

McCarrison (1924), McCarrison and Viswanath (1926) and Viswanath and Suryanarayana (1927) reported that seeds of plants grown on organic manures possess better nutritive value than crops grown with chemical fertilisers or with no manure. Using the onset of paralysis in rats as the test, McCarrison pointed out that cattle manure has been the best. Rowlands and Wilkinson (1930) found that grass seeds from soils manured with farmyard manure had greater amounts of vitamins than those grown on soils with artificial manures. Tallarico (1931) observed that turkeys fed on grains from lands fertilised with cattle manure showed more resistance to disease and even those that contracted diseases were less seriously affected. The recovery was found to be more rapid with a less number of fatal cases.

On the other hand, several workers have reported that organic manures have little or no characteristic effect on the vitamin content of plants. Harris (1934) employing bradycardia method of assay found that samples of wheat from plots at Rothamsted Experimental Station which had been treated with different types of manures did not show any striking variations in their vitamin B_1 content. Leong (1939) also reported that vitamin B_1 potency of wheat and barley was not significantly influenced by soil treatment. Scheunert and his associates (1936, 1937, 1939, 1940) carried out extensive investigations on the effect of animal manures and mineral fertilisers on the vitamin content of cereal grains and vegetables and showed that the type of fertiliser treatment had little or no effect on the thiamine, vitamin A and vitamin C content in plants.

In view of the controversial nature of the problem, the present investigation was undertaken with groundnut. Groundnut being rich in vitamin B_1, any slight variation in the vitamin as affected by manurial treatment can be accurately measured by chemical methods. The results of many

of the investigators referred to above were obtained by biological assays which are not sufficiently accurate to detect any minor changes in the vitamin B_1 content of the samples. Further, the differentiation of the B group of vitamins had not gone so far during the earlier period (1926).

Experimental

The cultural experiments were carried out in the Mysore Government Agricultural Farm, Hebbal, in dry land areas.

The land was ploughed twice and the lumps were broken. The plot was levelled and divided into 25 plots of the same area (area of each plot being 2 cents). It is a randomised square block. The cultural operations before and after sowing were done uniformly and at the same time on all plots. The sowing was done on 5th August 1946. The variety used was $H.G_1$ a popular variety in this locality. Same amount of seed was used in all plots. Sowing was done in lines (25 rows in each plot). Harvesting was done on 19th November 1946. The plants at the edges of plots were not included in the harvest. The pods were dried in the sun till constant weight was obtained. The results of the produce are presented in Tables I and II.

Plan of the Experiment

Five treatments and five replicates for each treatment.

Area of each plot	..	..	2 cents.
Total area	..	..	$25 \times 2 = 50$ cents.

The General Scheme of Manuring

Treatment	Manurial treatment
A	4 cartloads of cattle manure per acre (2 tons per acre).
B	6 cartloads of cattle manure per acre (3 tons per acre).
C	*Artificial manure.* Ammonium sulphate 45 lbs. per acre. Superphosphate 130 lbs. per acre. Muriate of potash 45 lbs. per acre.
D	Ammonium sulphate 67 lbs. per acre. Superphosphate 195 lbs per acre. Muriate of potash 67 lbs. per acre.
E	Control (no manure).

TABLE I

Showing yields of Groundnut-plants (green) and Pods from Plots differently treated

Treatments (average of 5 plots)	Number of plants	Weight of green plants in lbs.	Weight of pods in lbs.	Weight of pods per acre in lbs.
A Cattle manure ..	627	40·2	13·8	690·0
B do ..	692	51·3	15·9	795·0
C Artificial manure ..	632	43·9	14·1	705·0
D do ..	681	50·9	17·4	870·0
E No manure (Control) ..	489	22·8	8·6	430·0

Analyses of Seed for Vitamin B_1 Content from Plots differently treated

Preparation of material.—The material for vitamin B_1 analyses was prepared in the same way as described before in Part I, of the series.

The results of analyses are given in Table II.

TABLE II

Vitamin B_1 Content of Seeds grown in Plots differently manured

Treatment	Fat content per cent.	Vitamin B_1 content	
		Micrograms/gm. of defatted flour	I.U. per gram of defatted flour
A Cattle manure ..	44·5	14·9	4·97
B do ..	43·9	14·6	4·87
C Artificial manure ..	44·2	14·8	4·93
D do ..	44·6	14·9	4·97
E No manure (Control) ..	44·5	14·6	4·87

DISCUSSION

The results in the above table show that vitamin B_1 content of groundnut is not influenced by soil treatment. The extensive data of practical nature obtained so far on the above subject was summarised by Schopfer (1943) in the following way, " the vitamin content, especially A, B and C of a plant is a function of its development, at a given stage of growth, the content being at maximum. This content represents a biological constant characterising a given race. In large measure, it is practically independent of the nature of fertiliser utilised. Under extreme condition, a diminution may occur, but this is a result of a disturbed metabolism. The only available

method in altering the vitamin content of food plants is the selection and hybridization of races particularly rich in vitamins". The influence of variety on the vitamin B_1 content of groundnut was shown in Part I of the series. The vitamin content of a plant is a character which is determined genetically. It can more easily be influenced by genetical means than by chemical means such as fertilisers.

Summary

The groundnut (HG_1) grown in plots with various manurial treatments was analysed for vitamin B_1 content by thiochrome method. No differences in vitamin B_1 content were noticed, the relative values being as follows:

(A)	Cattle manure ..	14·9 micrograms/gm.	of defatted flour
(B)	„	14·6 „	„
(C)	Artificial manure ..	14·8 „	„
(D)	.. .. ..	14·9 „	„
(E)	No manure	14·6 „	„

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References

Harris, L. J. .. *J Agri. Sci.*, 1934, **24**, 410.

Leong, P. C. .. *Biochem. J.*, 1939, **33**, 1397.

McCarrison, R. .. *Brit. Med. Jour.*, 1924, **1**, 567.

——— and Viswanath, B. .. *Indian Jour. Med. Res.*, 1926, **14**, 351.

Rowlands, M. J. and Wilkinson, B. .. *Biochem. J.*, 1930, **24**, 199.

Schopfer, W. H. .. *Plants and Vitamins*—Chronica Botanica Co., Walthane, Mass, 1943.

Scheunert, A. and Schieblich, M. .. *Biederman's Zentbl.*, B, 1936, **8**, 120.

——— and Wagner, K. H. .. *Biochem. Ztschr*, 1937, **295**, 107.

——————— .. *Ibid.*, 1939, **303**, 200.

——————— .. *Ibid.*, 1939, **303**, 208.

——————— .. *Ibid.*, 1939, **303**, 268.

———, Reschke, J. and Kohlemann, E. *Ibid*, 1940, **305**, 1.

Tallarico, G. .. *Atti. Akad. Lineci.*, 1931, **13**, 294.

Viswanath, B. and Suryanarayana, M. .. *Me. Dept. Agr. Chem. Series*, 1927, **9**, 85.

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FOOD AND FEEDING HABITS OF THE FISHES OF THE GULF OF MANAAR

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I. Introduction

One of the more important problems of fishery research is the investigation of the nutrition of fishes in order to understand the qualitative and quantitative connections between them and their food organisms. This provides valuable data not only for the determination of the food chains, but also about the shoaling and migratory habits of fishes, thus foretelling the prospects for commercial fisheries on the coast. Marine fish-farming cannot be undertaken successfully without a knowledge of the food and feeding habits of the fish under culture, because fishing industry can be brought into line with other industries only when a detailed knowledge of the life-histories of the fishes and their food organisms is available.

II. Historical Resume

Not much is known hitherto about the nutrition of fishes in Indian seas. Except for some stray remarks by Day (1873, 1884, 1888 and 1889), Thurston (1887) and Thomas (1897), observations on the food and feeding habits were not undertaken until the two Marine Biological Stations were established by the Madras Fisheries Department in West Hill (Malabar Coast) and Krusadai Island (Gulf of Manaar) in 1921 and 1930 respectively. Since then, food and feeding habits have been studied in *Sardinella longiceps* (Hornell and Nayudu, 1924; Devanesan, 1943), *Sardinlla gibbosa*

TABLE I

S. No.	Species	No. of specimens examined	Range of sizes of fish in cms.	Stomach contents							Feeding habits
				Fish	Crustacea	Molluscs	Worms	Phyto & Zoo plankton	other groups	Debris	
1	*Carcharinus melanopterus*, Quoy and Gaim	52	45–150	*Epinephelus sp. Therapon jarbua, Leiognathus sp. Sillago sihama.*	*Panæus sp. Squilla sp.*	..	..	..	..	..	Carnivorous. Actively predaceous at surface and midwater
2	*Galeocerdo tigrinus*, Mull & Henle	1	250	*Hilsa toli. Charcharinus sp.* Upper jaw of *Pristis sp.*	..	..	..	..	..	..	do
3	*Stegostoma tigrinum* (Gmel.)	3	80–210	..	..	*Xancus pyrum, Natica sp.*	..	..	..	..	Carnivorous. Actively predaceous. Bottom feeder
4	*Narcine timlei* (Bl. Schn.)	2	32–44	*Sillago sihama. Gobius sp. Goboides sp.*	..	..	..	..	..	..	do
5	*Trygon uarnak* (Forskal)	17	25–90	*Therapon jarbua. Gerres oyena.*	*Penæus sp.*	Broken shells (unidentifiable)	*Lepionotus sp., Nereis sp., Perinereis sp., Marphysa sp.*	..	..	..	Omnivorous. Feeds at bottom and midwater

6	*Chanos chanos* (Forskal)	400	12–120	..	..	..	..	*Coscinodiscus*, *Fragilaria*, *Nitzschia*, *Pleurosigma*, *Rhizosolenia* *Thalassiothrix*, *Trichodesmium*, *Oikopleura*, *Frittilaria*, *Copepods*, Larval bivalves, *Leucifer*, *Mysis*, *Spiratella*, & Fish eggs and larvæ	..	..	Plankton feeder
7	*Chirocentrus dorab* (Forskal)	119	32–59	*Sardinella gibbosa* *Engraulis sp.*, *Stolephorus sp.*, *Dussumieria hasseltii*, *Trichiuris savala*.	..	..	..	..	..	..	Carnivorous. Actively predaceous at surface and midwater
8	*Sardinella gibbosa* (Cuv. & Val.)	1000	5–16	..	..	..	..	*Coscinodiscus*, *Detonula*, *Fragilaria*, *Rhizosolenia*, *Thalassiothrix*, *Trichodesmium*, *Foraminifers*, *Sagitta spp.*, Larval bivalves, *Creseis acicula* *Spiratella spp.*, Nauplius larvæ, Zœa larvæ, Megalopa larvæ, *Copepods*, *Leucifer*, *Rhopalophthalmus*, Fish larvæ.	..	..	Plankton feeder

TABLE I—(*Contd.*)

S. No.	Species	No. of specimens examined	Range of sizes of fish in cms.	Stomach contents							Feeding habits
				Fish	Crustacea	Molluscs	Worms	Phyto & Zoo plankton	other groups	Debris	
9	*Pellona bracysoma*, Bleeker	41	11–28	..	*Acetes sp.*, *Leander sp.*	..	..	*Biddulphia*, *Nitzschia*, *Rhizosolenia*, *Thalassiothrix*, *Copepods*, *Rhopalophthalmus*, *Amphipods*.	Sea weed (*Sargassum*)	..	Plankton feeder. Sometimes browsing on floating weeds
10	*Hilsa toli* (Cuv. & Val.)	8	18–24	..	*Acetes sp.* *Leander sp.*, *Caridina sp.*	..	..	Crab Zœa, Stomatopod larvæ.	..	..	Feeds mainly on macro-plankton
11	*Stolephorus indicus* (V. Hasset)	110	4–14	..	..	..	..	*Coscinodiscus*, *Thalassiothrix*, *Copepods*, *Leucifer*, *Rhopalophthalmus*.	..	..	Plankton feeder
12	*Dussumieria hasseltii*, Bleeker	1500	9–20	..	..	..	..	*Coscinodiscus*, *Rhizosolenia*, *Thalassiothrix*, *Trichodesmium*, *Copepods*, *Rhopalophthalmus*. *Leucifer*, Crab zœa, Megalopa larvæ, *Creseis acicula*, *Spiratella*, Larval bivalves, *Sagitta spp.*	..	..	Plankton feeder

13	*Anodontostoma chacunda* (Bloch)	131	16–24	..	..	..	..	*Trichodesmium*, *Copepods*, Larval bivalves, Fish eggs and larvæ.	..	..	Zoo-plankton feeder
14	*Anodontostoma nasus* (Bloch)	5	17–18	..	..	..	..	*Copepods*, Larval bivalves, *Spiratella sp.*	..	..	do
15	*Engraulis dussumierii* (Cuv. & Val.)	50	9–18	..	..	..	..	Larval bivalves, *Spiratella sp.*, *Copepods*, *Foraminifera*.	..	..	do
16	*Arius thalassinus* (Rupp.)	10	18–100	..	*Penæus sp.*, *Oxypod crab*	*Sepia*., Broken bivalve shells (*Mytilus*, *Pteria*, *Cardita* & *Cardium*)	..	..	Pieces of skin of *Enhydra* sp. with *Chelonobia testudinaria*. *Holothuria*.	Cycloid & ctenoid Fish-scales, Mud.	Omnivorous. Feeds at the surface; also browses at the bottom.
17	*Arius sona* (Ham.)	19	17–33	*Leiognathus*.	*Penæus sp.* *Appendages of crabs*	..	..	..	..	Fish-scales, (Cycloid).	Carnivorous. Predaceous at surface and mid-water
18	*Plotosus arab* (Forskal	3	14–16	..	*Appendages of crabs*.	Broken shells of bivalves (*Arca* & *Donax*).	..	..	..	..	Carnivorous. Feeds at bottom
19	*Saurus indicus*, Day.	26	17–27	*Stolephorus*.	*Penaeus sp.*	..	..	..	..	..	Carnivorous. Actively predaceous at surface.
20	*Belone strongylura*, v. Hasset.	27	30–44	*Stolephorus*.	*Acetes sp.*, *Penæus sp.*	..	..	:	..	..	do
21	*Hemiramphus georgii* (Cuv. & Val.)	24	26–29	..	..	..	..	*Copepods*, *Rhopalphthalmu*. Larval bivalves.	..	..	Plankton feeder

TABLE I—(*Contd.*)

S. No.	Species	No. of specimens examined	Range of sizes of fish in cms.	Stomach contents							Feeding habits
				Fish	Crustacea	Molluscs	Worms	Phyto & Zoo plankton	other groups	Debris	
22	*Cypsilurus bahiensis* (Razani)	30	17–21	..	..	..	..	*Coscinodiscus*, *Foraminfera*, *Biddulphia*.	..	..	Plankton feeder
23	*Mugil waigiensis*, Quo. & Gam.	296	10–45	..	..	..	Moults of *Nereis*, *Perinereis* & *Marphysa*	*Bacteriastrum*, *Biddulphia*, *Coscinodiscus*, *Detonula*, *Fragilaria*, *Hemidiscus*, *Navicula*, *Nitzschia*, *Pleurosigma*, *Rhizosolenia Thalassiothrix*, *Trichodesmium*, *Copepods*, *Ostracods*, *Spiratella sp.*, *Foraminifera* Larval bivalves.	Filaments of *Cladophora*, *Ceramium*, & *Polysiphonia*.	Mud.	Mainly a plankton feeder. Occasionally browses at bottom and on weeds
24	*Mugil trochelii*, Bleeker.	293	10–35	..	..	..	Moults of *Marphysa*. & *Perinereis*	*Chætoceras*, *Coscinodiscus*, *Fragilaria*, *Navicula*, *Nitzschia Pleurosigma*, *Rhizosolenia*, *Thalassiothrix*, *Tintinus*. *Naunlius larva* & *Copepods*.	Filaments of *Polysiphonia* & *Cladophora*.	Sand	do

25	*Mugil seheli* (Forskal)	141	9–21	..	..	..	..	*Bacteriastrum*, *Biddulphia*, *Coscinodiscus*, *Fragilaria*, *Nitzschia*, *Pleurosigma*, *Rhizosolenia*, *Thalassiothrix*, *Triehodesmium*.	..	..	Plankton feeder
26	*Sphyraena obtusata* (Cuv. & Val.)	51	17–45	*Sardinella gibbosa*, *Leiognathus sp.*, *Stolephorus sp.*, *Mugil troschelii*, *Leptocephalus larva*.	..	*Sepia*	..	..	..	..	Carnivorous. Actively predaceous at surface and midwater
27	*Polynemus heptadactylus* (Cuv. & Val.)	2	16–17	*Stoleporus sp.*, *Sardinella gibbosa*.	*Penæus sp.*	..	..	..	..	..	do
28	*Polynemus indicus*, Shaw	20	13–20	..	*Penæus sp.*, *Acetes sp.*	..	..	..	..	..	do
29	*Polynemus tetradactylus*, Shaw.	8	12–18	..	*Penæus sp.* *Acetes sp.*	..	..	..	..	..	do
30	*Elacate nigra* (Bloch)	1	47	..	*Neptunus pelagicus*.	..	..	..	..	..	do
31	*Cybium commersonii*, Lacep.	12	21–43	*Stolephorus sp.*, *Dussumieria hasseltii*.	..	*Sepia*	..	..	..	..	do
32	*Rastrelliger kanagurta*, Rupp.	80	22–26	..	..	..	..	*Asterionella*, *Coscinodiscus*, *Pleurosigma*, *Thalassiothrix*, *Copepods*, *Cresis acicula*, *Mysis*, *Larval bivalves*, *Spiratella sp.* Fish eggs and larvæ.	..	..	Plankton feeder

TABLE I—(*Contd.*)

S. No.	Species	No. of specimens examined	Range of sizes of fish in cms.	Stomach contents							Feeding habits
				Fish	Crustacea	Molluscs	Worms	Phyto & Zoo plankton	other groups	Debris	
33	*Trichiuris savala* (Cuv. & Val.)	24	41–85	*Sardinella gibbosa*, *Dussumieria hasseltii*, *Leiognathus bindus*, & *Lactarius lactarius*.	..	..	..	..	..	..	Carnivorous. Actively predaceous at surface and midwater
34	*Caranx hippos* (Linn.)	28	13–32	*Leiognathus sp.*, *Stolephorus sp.*	*Penæus sp.*	..	..	..	..	..	do
35	*Caranx sansum* (Forskal)	8	9–22	*Stolephorus sp.*	*Acetes sp.*, *Penæus sp.*	..	..	..	..	..	do
36	*Mene maculata* (Bl. Schn.)	69	22–26	..	..	..	..	*Coscinodiscus*, *Rhizosolenia*, *Thalassiothrix*, *Copepods*, *Crab-zoe*, *Cresis acicula*, Larval bivalves *Leucifer hansenii*, *Rhopalophthalmus*, *Spiratella sp.*	Filaments of *Ectocarpus*, & *Polysiphonia*.	..	Plankton feeder, occasionally browsing on floating vegetation
37	*Lactarius lactarius* (Cuv. & Val.)	3500	11–26	*Sardinella gibbosa*, *Engraulis indicus*, *Stolephorus sp.*, *Sciaena glauca*, *Leiognathus sp.*, *Trichiuris savala*, & *Pseudorhombus arsius*.	*Acetes sp.*	..	..	..	..	..	Carnivorous. Actively predaceous at surface and midwater

38	*Leiognathus bindus* (Cuv. & Val.)	1200	4–16	..	..	..	..	*Coscinodiscus*, *Rhizosolenia*, *Nitzschia*, *Thalassiothrix*, *Fragilaria*, *Pleurosigma*, *Biddulphia*, *Detonula*, *Copepods*, *Rhopalophthalmus*, *Leucifer*, *Megalopa larva*, Larval bivalves, *Spiratella sp.*, Fish eggs.	..	..	Plankton feeder
39	*Lates calcarifer* (Bloch)	16	16–28	*Mugil seheli*, *Chanos chanos*, *Elops indicus*	*Penæus sp.*, *Squilla sp.*	Broken bivalve shells (*Arca* & *Mytilus*)	..	..	Seaweed (*Enhalus*)	Sand	Carnivorous. Predaceous at mid-water. Sometimes browses on rocks and weeds
40	*Epinephelus bænack* (Forskal)	61	19–38	*Therapon jarbua.*	*Penæus sp.* Appendages crabs.	..	..	..	Seaweed (*Halimeda*)	..	Carnivorous. Active at bottom
41	*Lutjanus lineolatus* (Rupp.)	63	10–23	..	*Alpheus sp.*, *Squilla sp.*, *Lysiosquilla*, & Appendages of crabs.	..	..	..	..	Bits of dead coral	Carnivorous. Actively predaceous, at mid-waters. Sometimes browses on corals
42	*Diagramma crassispinum*, Rupp.	29	16–22	..	*Acetes sp.*, *Penæus sp.*	..	*Nereis*, *Marphysa*	..	..	..	Omnivorous. Feeds at mid-water and bottom
43	*Therapon jarbua* (Forskal)	281	9–16	*Engraulis sp.*, *Gnobius sp.*, *Young tetradon.*	*Gonodactylus sp.*, *Alpheus sp.*	*Sepia*	*Marphysa*, *Perinereis*	..	*Balanoglosus*	Sand, Fish-scales, (Cycloid).	Omnivorous. Feeds at all levels
44	*Diagramma punctatum* (Cuv. & Val.)	4	20–38	..	*Metagrpsus sp.*, *Ocypoda sp.*	*Potamides*	*Marphysa*	..	Seaweeds (*Halimedi*)	Fish-scales, Sand	Omnivorous. Feeds mostly at bottom

TABLE I—(*Contd.*)

S. No.	Species	No. of specimens examined	Range of sizes of fish in cms.	Stomach contents							Feeding habit
				Fish	Crustacea	Molluscs	Worms	Phyto & Zoo plankton	Other groups	Debris	
45	*Chrysophrys datnia* (Ham.)	150	12–27	..	*Penæus sp., Acetes sp., Ocypoda sp.*	Bivalve & gastropod shells (unidentifiable)	*Marphysa, Nereis*	..	..	Fish-scales, (cycloid)	Carnivorous
46	*Synagris japonicus* (Bloch)	34	13–19	..	Appendages of crabs.	Broken bivalve shells (*Modiolus* & *Pteria*)	..	*Coscinodiscus, Rhizosolenia, Thalassiothrix, Copepods, Leucifer, Rhopalophthalmus, Spiratella sp.*	Small *ophiuroids.*	Sand	Mostly plankton feeder; Sometimes browsing at bottom
47	*Gerres filamentosus* (Cuv. & Val.)	260	14–24	..	..	Fragments of bivalve shells (unidentifiable)	*Perinereis Marphysa, Polydora*	..	Seaweeds: *Halimeda, Sargassum.*	..	Omnivorous: browsing at bottom and among weeds
48	*Gerres oyena* (Forskal)	35	14–20	..	..	..	..	..	Seaweeds: *Halimeda, Gelidium.*	..	Browses among weeds
49	*Gerres abbreviatus,* Bleeker.	60	7–23	..	..	Bivalve shells (unidentifiable)	*Marphysa, Polydora*	..	Seaweed: *Halimeda.*	..	Omnivorous; browses at bottom and among weeds
50	*Upeneoides tragula* (Richardson)	125	13–15	*Leiognathus sp.*	*Acetes sp. Penæus sp.* Appendages of crabs	Bivalve shells (*Lithodomus*)	..	..	..	..	Carnivorous. Predaceous at mid-water and bottom

					Appendages of crabs	(*Lithodomus*)						
51	*Sciæna albida* (Cuv. & Val.)	126	13–19	*Stolephorus sp., Dussumieria hasseltii*, and young *Trichiuris savala.*	*Penæus Sp.*	Bivalve shells (*Mytilus, Pteria* & *Donax*)	..	..	..	Bits of test of sea-urchin	do
52	*Sciæna glauca*, Day.	64	14–19	*Leiognathus sp.*	*Penæus Sp.*	Spats of *Avicula sp.*	..	..	..	Seaweed : *Enhalus*	do
53	*Otolithus ruber* (Bl. Schn.)	57	16–32	*Leiognathus sp., Sardinella gibbosa, Engraulis sp., Stolephorus sp.* Young *Trichiuris savala.*	..	..	..	..	..	..	Carnivorous. Actively predaceous at surface and midwater
54	*Otolithus maculatus* (Cuv. & Val.)	19	15–24	..	*Penæus, Aeetes* & *Squilla.*	..	..	..	..	..	do
55	*Sillago sihama* (Forskal)	281	8–31	*Zonogobius sp.*	*Penæus sp. Ocypoda, Alpheus, Gonodactylus.*	..	*Marphysa, Perinereis, Nereis*	..	Seaweed : *Sargassum*, & *Enhalus, Balanoglossus.*	Sand	Omnivorous. Browses among corals and weeds
56	*Cirrhitichthys aureus* (Temm. & Schleg.)	17	28–32	..	*Thalamita sp. Phylira sp., Gonodactylus*	..	..	..	..	..	Carnivorous. Predaceous at bottom
57	*Tuethis vermiculata* (Cuv. & Val.)	28	17–35	..	..	..	..	..	Seaweeds : *Turbinaria, Sargassum, Halimeda, Acanthophora* & *Gracilaria.*	Cycloid Fish-scales Sand	Browsing among weeds
58	*Platycephalus insidiator* (Forskal)	11	16–24	..	*Alpheus, Gonodactylus*	..	..	..	..	..	Carnivorous. Predaceous at bottom
59	*Platyglossus hortulans* (Lacep.)	27	11–48	..	*Ocypoda, Gonodactylus*	..	..	..	Seaweed : *Turbinaria.*	..	Omnivorous . browsing on rocks and weeds

(Devanesan, 1932; Chacko, 1946), the Indian Mackerel *Rastrelliger kanagurta* (Devanesan and John, 1940; Chidambaram, 1944), *Leiognathida* (Chacko, 1944), *Kowala thoracata* (Devanesan and John, 1941), *Lactarius lactarius* (Chacko, 1944), the Ribbon Fish *Trichiurus* sp. (Venkatraman, 1944), *Chanos chanos* (Chacko, 1945), Mullets, *Mugil* spp. (Chacko and Venkatraman, 1945), the Tiger Shark, *Galeocerdo* sp. (Chidambaram, 1945), the Rainbow Sardine, *Dussumieria hasseltii* (Devanesan and Chacko, 1944), certain fishes of the West Coast of the Madras Presidency (Chidambaram and Venkatraman, 1946), and in species of Sharks of the Madras Coast (Chidambaram and Menon, 1946; John and Somayaji, 1946). Observations on some other species of marine fish have also been recorded in papers dealing with plankton of the Madras Coast (Chidambaram, 1942; Devanesan, 1942; Devanesan and Chacko, 1944; Varadarajan and Chacko, 1943). Amongst other investigators on the nutrition of South Indian marine fishes. Job's work (1940) deserves special mention. The food of post-larval fishes, was studied by Gopinath (1942 and 1946); and the food of the Oil Sardine and Mackerel by John and Menon, 1942) at the Marine Biological Station, Trivandrum. Gnanamuthu (1943) in a paper on the Foraminifera of Krusadai Island, recorded several species of these protozoa from the intestinal contents of *Sardinella*, *Mugil*, *Hemirhamphus* and *Belone*, while Deraniyagala (1944) described the stomach contents of the Whale Shark, *Rhineodon typehus*.

III. Analysis of the Food

The food components and feeding habits of 59 species occurring in the Gulf of Manaar is tabulated in Table I.

IV. Discussion

The foregoing analysis of food shows that most of the fishes of the Gulf of Manaar are carnivorous, omnivorous and plankton-feeding. The main carnivorous species are the Shark (*Carcharinus melanopterus*), the Tiger Shark (*Stegostoma tigrinum*), the Sabre-fish (*Chirocentrus dorab*), the Lizard-fish (*Saurus indicus*), the Gar-fish (*Belone strongylura*), the Barracuda (*Sphyræna obtusata*), the Big-jawed Jumper (*Lactarius lactarius*) and the Jew-fish (*Otolithus ruber*).

A purely herbivorous feeding habit is not seen in any of the fishes. Even in *Teuthis vermiculata*, the Unicorn-fish, whose stomach and intestine are usually gorged with sea-weeds, fish scales were found, indicating thereby feeding on dead fish. It is however possible that fish scales are just entangled among the weeds.

A mixed diet was found in the Bhekti (*Later calcarifer*), the Rock-cod (*Epinephelus boenack*), the Squeaking Perch (*Theropon*), the Porgy (*Chrysophyrys datnia* and other fishes.

Plankton feeding was commonly met with among the sardines (*Pellona brachysoma, Anodontosoma chacunda* and *Dussumieria hasseltti*), the milk-fish (*Chanos chanos*), the Silver-belly (*Leiognathus bindus*) and the mackerel (*Rastrelliger kanagurta*). The Mullets (*Mugil waiguensis* and *M. troscheli*) supplement a diet of plankton with algal filaments and moults of bristle-worms. The latter may also be accidental inclusions which got entangled amongst algal filaments. In the stomach of one specimen of cat-fish (*Arius thalassinus*) were found one large entire specimen of a sea-cucumber (*Holothuria scabra*), five crabs, one prawn and abundant remains of crustacea. In the stomach of another specimen of the same species were found large bits of the skin of a sea snake, one turtle-barnacle (*Chelonobia testudinaria*), as well as appendages of crabs. Bivalve shells and cuttle fish (*Sepia*) were observed in other specimens.

The habit of selective feeding in some of the species is worth noting. Sea-weeds are eaten by *Teuthis vermiculata, Platyglossus hortulanus* and several other fishes, the sea-weed *Halimeda* being more commonly eaten than any other. Of the plankton-feeders, *Sardinella gibbosa, Anodontostoma chacunda, Chanos chanos* and *Mugil waigiensis* feed upon the alga *Trichodesmium*. Foramanifera were recorded in *Sardinella gibbosa* and in the Anchovy, *Engraulis dussumieri*; and Arrow-worms (*Sagitta* spp.) from the stomachs of *Sardinella gibbosa* and *Dussumieria hasseltii*. The habit of eating fish eggs and larvæ is found in *Anodontostoma chacunda, Chanos chanos* and *Rastrelliger kanagurta*. Some fishes feed on bristle-worms and what appear to be moults of bristle-worms; for example, *Therapon jarbua, Chrysophrys datnia* and the Whiting, *Sillago sihama* on *Nereis, Perinereis* and *Marphysa*. Bivalve and gastropod shells are mostly found in the stomach of the Sting Ray (*Trygon uarnak*), *Arius thalassinus*, the Silver-perches (*Gerres filamentosus* and *G. abbreviatus*), and *Chrysophrys datnia*. In the stomach of one specimen of *Stegostoma tigrinum* examined, there were 14 opercula of the Sacred Chank, *Xanous pyrum*. Crabs and prawns form the diet of many fishes, the important ones being *Carcharinus melanopterus, Trygon uarnak, Arius thalassinus, Epinephelus boenak, Lutianus lineolatus, Therapon jarbua, Elacate nigra* and *Sillago sihama*. There are many fishes feeding on other fishes. *Chirocentrus dorab, Lactarius lactarius* and *Otolithus ruber* are the chief enemies in the fisheries of *Sardinella gibbosa, Engraulis* spp. *Leiognathus* spp., and *Stolephorus* spp. Fish scales were observed in the

stomachs of *Arius sona*, *Therapon jarbua*, *Chrysophrys datnia* and *Teuthis vermiculata*, and consequently in these, feeding on dead fishes is suspected.

V. Acknowledgments

I am indebted to Rao Sahib Dr. D. W. Devanesen, M.A., Ph.D., D.I.C., Assistant Director of Fisheries, Marine Biology, Madras, for his kind help and suggestion during this investigation. I am thankful to the Director of Industries and Commerce, Madras, for according necessary permission for the publication of this paper. My thanks are also due to Professor Beni Charan Mahendra for valuable suggestions and criticism.

VI. Summary

The composition of the food of 59 species of marine fishes occurring in the Gulf of Manaar is recorded. Their feeding habits are discussed.

VII. Bibliography

Chacko P. I. .. "On the Bionomics of the *Leiognathidæ*," *Curr. Sci.*, 1944, **13**, 214.

———— .. "On the Bionomics of the Big Jawed Jumper, *Lactarius lactarius*," *ibid.*, 1944, **13**, 108.

———— .. "On the Food and Alimentary Canal of the Milk-Fish *Chanos chanos*," *ibid.*, 1945, **14**, 242–43.

———— .. "On the Bionomics of the Indian Sprat *Sardinella gibbosa*," *Journ. Bombay Nat. Hist. Soc.*, 1946, **46**, 407-08.

—— and Venkatraman, R. S. "On the Food of Mullets," *Curr. Sci.*, 1945, **14**, 79.

Chidambaram, K. .. "On the Alleged Inhibitory Influence of *Trichodesmium*," *ibid.*, 1942, **11**, 406–07.

———— .. "Food of the Indian Mackerel, *Rastrelliger kanagurta*, of the West Coast of the Madras Presidency," *ibid.*, 1944, **13**, 214.

———— .. "Note on the Food of the Tiger Sharks, *Galeocerdo* spp., of the Madras Coasts," *Journ. Bombay Nat. Hist. Soc.*, 1945, **45**, 247.

—— and Menon, M. D. .. "Investigations of the Shark Fishery of Madras Presidency," *Madras Fish. Bull.*, 1946, 1–18.

—— and Venkatraman, R. S. "Tabular Statements on the Natural History of Certain Marine Food-Fishes of the Madras Presidency—West Coast," *ibid.*, 1946, 1-26.

Day, F. .. *Report on the Sea Fish and Fisheries of India and Burma*, Calcutta, 1873.

———— .. "The Food of Fishes," *Fish. Exhi, Lit.*, 1884, **6**, 265–300.

———— .. *The Fishes of India*, London, 1888.

———— .. *The Fauna of British India—Fishes*, London, 1889, **1 & 2**.

Deraniyagala, P. E. P. .. "Some Whale Sharks and Sun Fishes Captured off Ceylon," *Journ. Bombay Nat. Hist. Soc.*, 1944, **44**, 426–30.

Devanesen, D. W. .. "A Note on the Food and Feeding Habits of *Sardinella gibbosa*," *Journ. Madras Univ.*, 1932, **4**, 159–64.

———— .. "Plankton Studies in the Fisheries Branch of the Department of Industries and Commerce, Madras," *Curr. Sci.*, 1942, **11**, 142–43.

———— .. "A Brief investigation into the Causes of the Fluctuation of the Annual Fishery of the Oil Sardine of Malabar, *Sardinella longiceps*, Cuv. & Val., Determination of its Age and an Account of the Discovery of its Eggs and Spawning Ground," *Madras Fish. Bull.*, 1943, **28**, 1–38.

—— and Chacko, P. I. .. "Balanoglossus as Food of Fishes," *Curr. Sci.*, 1942, **11**, 242–43.

———— .. "On the Fluctuation of a Few Typical Organisms in the Sea around Krusadai Island, Gulf of Manaar, for the quinquennium, 1936–37 to 1940–41," *Proc. 31st Indian Sci. Cong.*, 1944, **3**, 116–17.

———— .. "On the Bionomics of the Rainbow Sardine, *Dussumieria hasseltii*," *Proc. Nat. Inst. Sci. India*," 1944, **10**, 143–45.

—— and John, V. .. "On the Natural History of *Rastrelliger kanagurta* with special reference to its spawning season and eggs," *Curr. Sci.*, 1940, **9**, 462–64.

———— .. "On the Natural History of *Kowala thoracta* with special reference to its gonads and eggs," *Rec. Indian Mus.*, 1941, **43**, 215–16.

Gnanamuthu, C. P. .. "The Foraminifera of Krusadai Island," *Bull. Madras Gov. Mus.*, 1943, **1**, 1–21.

Gopinath, K. .. "Distribution and Feeding of the Post-larval Fishes of the Trivandrum Coast," *Curr. Sci.*, 1942, **11**, 337–38.

———— .. "Preliminary Observations on the Food of Post-larval Fishes," *Proc. 32nd Indian Sci. Cong.*, 1946, **3**, 130–31.

Hornell, J. and Nayudu, M. R... "A Contribution to the Life-History of the Indian Sardine," *Madras Fish. Bull.*, 1924, **17**, 129–97.

Job, T. J. .. "An Investigation on the Nutrition of the Perches of the Madras Coast," *Rec. Indian Mus.*, 1940, **42**, 289–364.

John, C. C. and Menon, M. A. S. "Food and Feeding habits of the Oil Sardine and Mackerel," *Curr. Sci.*, 1942, **11**, 243–44.

John, V. and Somayaji, A. .. "A Report on the Deep Sea Fishing Experiments conducted in 1945–46," *Madras Fish. Bull.*, 1946, 1–24.

Thomas, H. S. .. *The Rod in India*, London, 1897.

Thurston, E. .. "Preliminary Report on the Marine Fauna of Rameswaram and the Neighbouring Islands," *Madras. Govt. Mus. Sci. Ser.*, 1887, **1**, 22–27.

Varadarajan, S. and Chacko, P. I. "On the Arrow Worms of Krusadai Island," *Proc. Nat. Inst. Sci. India*, 1943, **9**, 245–48.

Venkatraman, R. S. .. "The Food of the Ribbon Fish, *Trichiurus* spp," *Curr. Sci.*, 1944, **13**, 239.

A CONTRIBUTION TO THE EMBRYOLOGY OF *DROSERA BURMANNI* VAHL.

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In the literature on the Droseraceæ, there is available some information on details of reproductive organs and embryology (Peters, 1898), describing the reproductive organs and embryology of *Drosera*, stated that no parietal cells are formed, whilst Rosenberg (1899) and Lang (1901) found that parietal cells are formed. Pace (1912) has confirmed the latter findings, but Warming (1913) does not find any parietal cells being formed during development. Smith (1929) has worked out the life-history of *Dionaea muscipula*, and finds that parietal cells are not formed during development. Recent work on the embryology of Droseraceæ is by Soueges (1936) on *Drosera rotundifolia* L.

Drosera Burmanni Vahl., which flowers all the year round and is found in marshy places near tanks round about Bangalore, is selected as the material for the present study. The material was fixed in Allen's modified Bouin. Sections were cut from 4 μ to 6 μ and stained in Heidenhain's iron-alum-hæmotoxylin.

Organogeny of the Flower

The different floral parts take their origin in acropetal succession, *viz.*, the sepals, the petals, the stamens and finally the carpels.

Microsporangium

The wall of the young anther is made up of four layers—the epidermis, the endothecium which develops fibrous thickenings at maturity, a single middle layer, and the innermost layer, the tapetum, with cells uninucleate in the beginning and becoming binucleate later (Fig. 1). The microspore-mother cells after undergoing reduction divisions form microspore quartets and show either bilateral or tetrahedral arrangement (Figs. 2 and 3). The individual microspores of the quartet do not separate but remain together as a unit so that they may be appropriately designed as " compound grains " as in some orchids. Even pollen tubes are put forth in the same condition of the quartet. The wall of the individual grains of the unit is thrown out

into plaits at the proximal end where the four microspores meet and the distal wall of the exine shows characteristic sculpturing (Fig. 5). When fully mature the pollen grains are two-celled (Fig. 6). Occasionally, in some anthers, the pollen grains were found to put forth pollen tubes, which feature possibly suggests a tendency towards cleistogamy.

During the study of meiosis in the microspore-mother cells, the number of chromosomes was determined to be ten (Fig. 4). Rosenberg (1904, 1909) finds in *Drosera rotundifolia* and *D. longifolia* the number of chromosomes to be ten and twenty respectively. Levine (1916) finds the number of chromosomes to be ten in *Drosera filiformis*.

Megasporangium and the Development of the Embryo-sac

The ovary is unilocular with parietal placentation. Ovules take their origin on the slightly projecting placentæ. Of the two integuments, the outer one which arises later is shorter than the inner which organises the micropylar canal (Fig. 13). Both the integuments are two cells thick, but the number of cell layers of the inner one at the micropylar end is three to four. In older ovules, the outer layer of the inner integument and the inner layer of the outer integument are crushed.

The archesporial cell is single and hypodermal and is differentiated even as the integuments are being formed (Fig. 7). Rarely, two to three archesporial cells were seen (Fig. 8). The archesporial cell gives rise to a single parietal cell which further divides by a vertical wall, and the megaspore mother cell (Fig. 9).

The megaspore mother cell undergoes the usual meiotic divisions and gives rise to a T-shaped tetrad (Figs. 10 and 11). The upper three megaspores degenerate, while the lowermost develops into a typical eigth-nucleate embryo sac (Figs. 12 and 13). The synergids are shorter than the egg and pear shaped, sometimes taking a darker stain than the egg. The polar nuclei fuse early before fertilization and the secondary embryo sac nucleus lies immediately below the egg. The antipodals are organised as definite cells and are slightly triangular in shape. Invariably they show signs of degeneration by the time the embryo sac is fully organised but sometimes persist as late as the first division of the endosperm nucleus (Figs. 13 and 14).

The fully formed embryo-sac does not occupy more than one-third of the nucellus. The nucellus is peculiarly differentiated at this stage—(1) The layer adjacent to the inner integument which at the same time surrounds the embryo-sac becomes elongated transversely and shows conspicuously vacuolated cytoplasm and (2) linearly elongated cells with darkly staining cytoplasm found in the central region below the embryo sac (Fig. 13).

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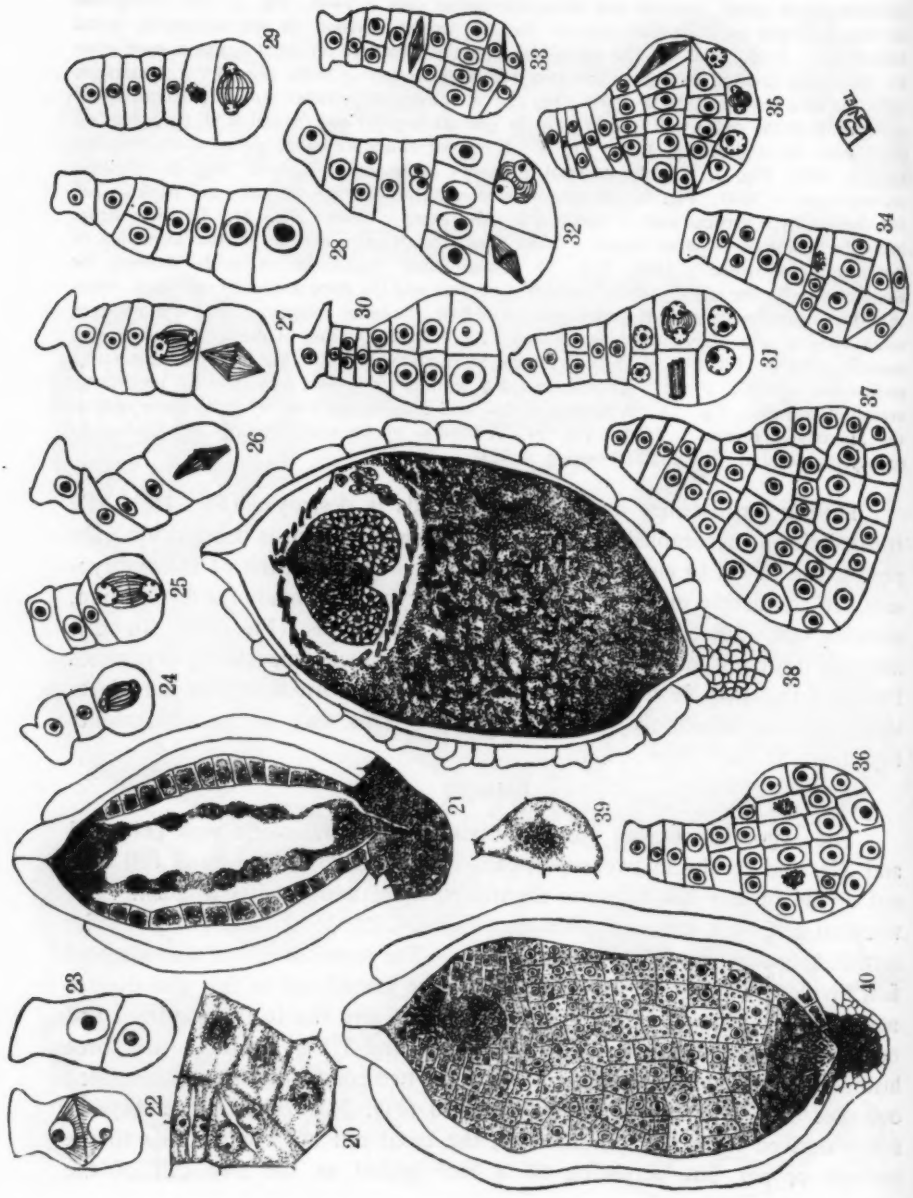
FIGS. 1–19. Fig. 1. Portion of a transverse section of a young anther showing the epidermis, wall layers, tapetum and micropore-mother cells × 1440. Fig. 2. The metaphase spindles arranged in a parallel manner for the second division in the microspore tetrad formation × 3600. Fig. 3. The metaphase spindles arranged at right angles to each other for the second division in the microspore tetrad formation × 3600. Fig. 4. A metaphase plate showing ten bivalents × 3600. Fig. 5. A microspore quartet with the characteristic sculpturing of the outer wall (shown only for one microspore) and the union of the individual grains with its neighbour × 3600. Fig. 6. A quartet of mature pollen grains at the shedding stage × 3600. Fig. 7. The hypodermal primary archesporium × 3600. Fig. 8. Multiple archesporium × 3600. Fig. 9. Megaspore mother cell with parietal cell; the parietal cell has divided by a vertical wall × 3600. Fig. 10. Dyad × 3600. Fig. 11. Division figure in the formation of T-shaped tetrad × 3600. Fig. 12. Nuclear division in the formation of 8-nucleate embryo-sac × 3600. Fig. 13. Longitudinal section of an ovule showing the embryo-sac with the egg-apparatus, secondary nucleus and the three antipodal cells and transversely elongated nucellar cells; micropyle formed by the inner integument and the enlarged outer cells of the outer integument × 480. Fig. 14. I Division of the primary endosperm nucleus × 3600. Fig. 15. A stage in double fertilisation × 3600. Fig. 16. A degenerating embryo-sac × 3600. Fig. 17. An abnormal degenerating embryo-sac with 16 nuclei imperfectly organised × 3600. Fig. 18. A portion of the seed showing division of endosperm cells at the micropylar regions × 3600. Fig. 19. A portion of the seed showing the division of endosperm cells at the chalazal region × 3600.

During fertilisation, one of the synergids is destroyed (Fig. 15). The triple fusion is accomplished earlier than syngamy which is delayed upto the production of six to eight endosperm nuclei. Degeneration of the embryo-sacs are frequently met with, when the embryo sac is ready for fertilisation, showing the disorganised synergids and antipodals (Fig. 16). In one instance, an embryo-sac was found with sixteen nuclei imperfectly organised. Probably this may be due to the coalition of two normal embryo-sacs developed by two adjacently lying megaspores. This may be an abnormality Fig. 17).

EMBRYO

The zygote undergoes the first division by a transverse wall (Figs. 21 and 22), and results in two superposed cells (Fig. 23). The basal cell does not divide further but becomes slightly enlarged and persists as such. The terminal cell by a series of divisions (Figs. 24, 25, 26, 27 and 28) gives rise to a filamentous row of six to seven cells. The terminal cell of this filament first divides by a vertical wall (Fig. 29). The second cell to this also divides next by a vertical wall. The two pairs of cells give rise to the quadrant and octant stages (Figs. 30, 31, 32, 33, 34, 35, 36 and 37) giving rise to the three histogens. The mature embryo in which the two cotyledons are differentiated occupies only a small portion in the seed (Fig. 38). Thus the embryo is solely derived from the terminal cell, the basal cell not contributing to the embryo proper but persisting till a late period as the free cell of the suspensor.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FIGS. 20–40. Fig. 20. A portion of the seed showing the division of endosperm cells in the central portion × 3600. Fig. 21. Zygote with endosperm nucleus lining the wall of the embryo-sac × 3600. Figs. 22–37 Stages in embryogeny × 3600. Fig. 38. Longitudinal section of a mature seed showing the dicotyledonous embryo, rich contents and remnants of endosperm cells at the micropylar end × 640. Fig. 39. A metaphase plate showing thirty chromosomes in one of the endosperm cells × 3600. Fig. 40. Longitudinal section of a fairly advanced embryo with the differentiation of endosperm cells at the micropylar middle and chalazal regions × 640.

ENDOSPERM

The primary endosperm nucleus divides much earlier than syngamy is accomplished (Fig. 14). After many and repeated free nuclear divisions the endosperm tissue forms a lining along the periphery of the sac (Fig. 21). Thirty chromosomes were clearly counted during these divisions (Fig. 39). Subsequently the nuclear divisions proceed inwards so that the endosperm nuclei become somewhat loosely and evenly distributed throughout the embryo-sac. At a slightly later stage, some of the endosperm nuclei towards the chalazal end show a tendency to gather together with a larger quantity of cytoplasm. However, this does not develop into any special organ of the endosperm as has been reported in *Hypericum* by Swamy (1946).

Wall formation takes place centripetally from the micropylar end. At the mycropylar end particularly, the endosperm cells divide further (Fig. 18) and increase in bulk. These cells are smaller and arranged rather loosely. In the middle region, the endosperm cells are large and become stored with starch and rich contents (Figs. 40 and 20). At the chalazal end, the endosperm cells are smaller with an irregular outline and arranged in a compact mass (Fig. 19).

SUMMARY

1. The development of the anther is quite normal and the tapetal cells are bi-nucleate. The haploid chromosome number has been determined as ten. The individual microspores of the quartet do not separate but remain together as a unit and the individual pollen grain of the quartet is bi-nucleate at the shedding condition.

2. The nucellus is differentiated peculiarly into two distinct regions, (1) a region contiguous with the inner integument having transversely elongated cells with conspicuously vacuolated cytoplasm, (2) linearly elongated cells, with darkly staining cytoplasm formed as an axial strand below the embryo sac.

3. The primary archesporial cell divides to form a parietal cell. A T-shaped tetrad is formed and the chalazal megaspore develops into the monosporic eight-nucleate embryo-sac. The antipodals are organised as definite cells.

4. The first division of the zygote is transverse resulting in a basal cell and a terminal cell. The basal cell does not divide further and forms part of the suspensor. The terminal cell divides and takes part in the formation of the entire embryo.

5. The endosperm is free nuclear and distinguished into three regions in the mature seed. Thirty chromosomes have been counted in the endosperm cell.

Grateful acknowledgments are made to Prof. L. S. Dorasami, M.Sc. (Lond.), PH.D. (Lond.), Economic Botanist, for critical suggestions and encouragement during the course of this investigation.

LITERATURE CITED

1. Lang, F. X. .. "Untersuchungen uber Morphologie, Anatomie und samenentwicklung von Polypompholy und *Biblis gigantea*," *Flora*, 1901, **88**, 149–206.

2. Pace, L. .. "*Parnassia* and some allied genera," *Botanical Gazette*, 1912, **54**, 306–29.

*3. Peters, C. A. .. "Reproductive organs and embryology of *Drosera*," *Proc. Amer. Ass. Adv. Sci.*, 1898, 46–275.

*4. Rosenberg, O. .. *Physiologisch–cytologische untersuchungen uber* Drosera rotundifolia, 1899, 126, Pls. 2.

*5. ———— .. *Uber die Reductionsteilung eines* Drosera-*Bastardes* (Meddel Stockholms Hogskolas), 1904 *a*.

*6. ———— .. "Cytologische und morphologische studien an *Drosera longifolia* × *rotundifolia*," *Svenska, vetensk, Akad, Handlinger*, 1909 *a*, 43.

7. Levine, M. .. "Somatic and reduction divisions in certain species of *Drosera*," *Mem. N. York Bot. Garden*, 1916, **6**, 125–47.

8. Smith, C. M. .. "Development of *Dionaea muscipula*," *Botanical Gazette*, 1929, 508–530.

9. Soueges .. "Embryogenie des Droseracees-development de l'embryon chez le *Drosera rotundifolia* L." *C. R. Acad. Sc. cc.* II, p. 1457. 25 Fig. Seance D. W. 27 Avril, 1936 *f*.

10. B. G. L. Swamy .. "Endosperm in *Hypericum mysorense* Heyne," *Annals of Botany*, April 1946, N. S., **10**, No. 38.

* Citations from *Die zytologie der Blutenpflanzen*, by P. N. Schurhoff, Stuttgart, 1926, and *Embryologie der Angiospermen*, by K. Schnarf, Berlin, 1929.

MORPHOLOGY AND CYTOLOGY OF *POLYSIPHONIA PLATYCARPA* BOERGS.*

Preliminary Note

By M. O. P. Iyengar and M. S. Balakrishnan

Received February 22, 1949

The only species of *Polysiphonia* that has been worked out in detail both as regards its developmental morphology and cytology is *Polysiphonia violacea* (Yamanouchi, 1906 A; 1906 B). Since then, the same species and a few other species of the genus have been worked out by other authors as regards their developmental morphology (Connolly, 1911; Kylin, 1923, 1937; Grubb, 1925; Rosenberg, 1933), but the cytological details were not followed by these workers.

Polysiphonia platycarpa Boergs. occurs in plenty in the estuarine portion of the river Adyar at Madras and in the back-waters at Ennur eleven miles north of Madras, and was therefore taken up for a detailed investigation. This species showed several interesting features:

(i) The carpogonial branch in this species is unique in being three-celled (Fig. 1), while in all other members of the Rhodomelaceæ so far investigated it is four-celled.

(ii) The fusion of the fertilised carpogonium with the auxiliary cell is effected by a small connecting cell cut off by the carpogonium (Fig. 5 *Con. C*). A connecting cell is usually absent in the Rhodomelaceæ though it has been previously recorded in two members of the family, *viz.*, *Polysiphonia decipiens* (Connolly, 1911) and *Laurencia pinnatifida* (Kylin, 1923).

(iii) Yamanouchi (1906 B, pp. 420–423) has described an extraordinary type of reduction division in *Polysiphonia violacea* during tetraspore formation. According to him the heterotypic division in this alga stops at anaphase and the homeotypic division begins where the heterotypic division ends. His description of reduction division is as follows: "The spindle of the first mitosis is of very short duration. As soon as the chromosomes at the equatorial plate separate into two groups, the two spindles of the second mitosis suddenly appear and the first spindle can no longer be recognised.... The second mitosis of the tetraspore-mother-cell, therefore, follows so shortly after the first that there is no period between for the organisation of two resting nuclei." In other words, the later stages of the

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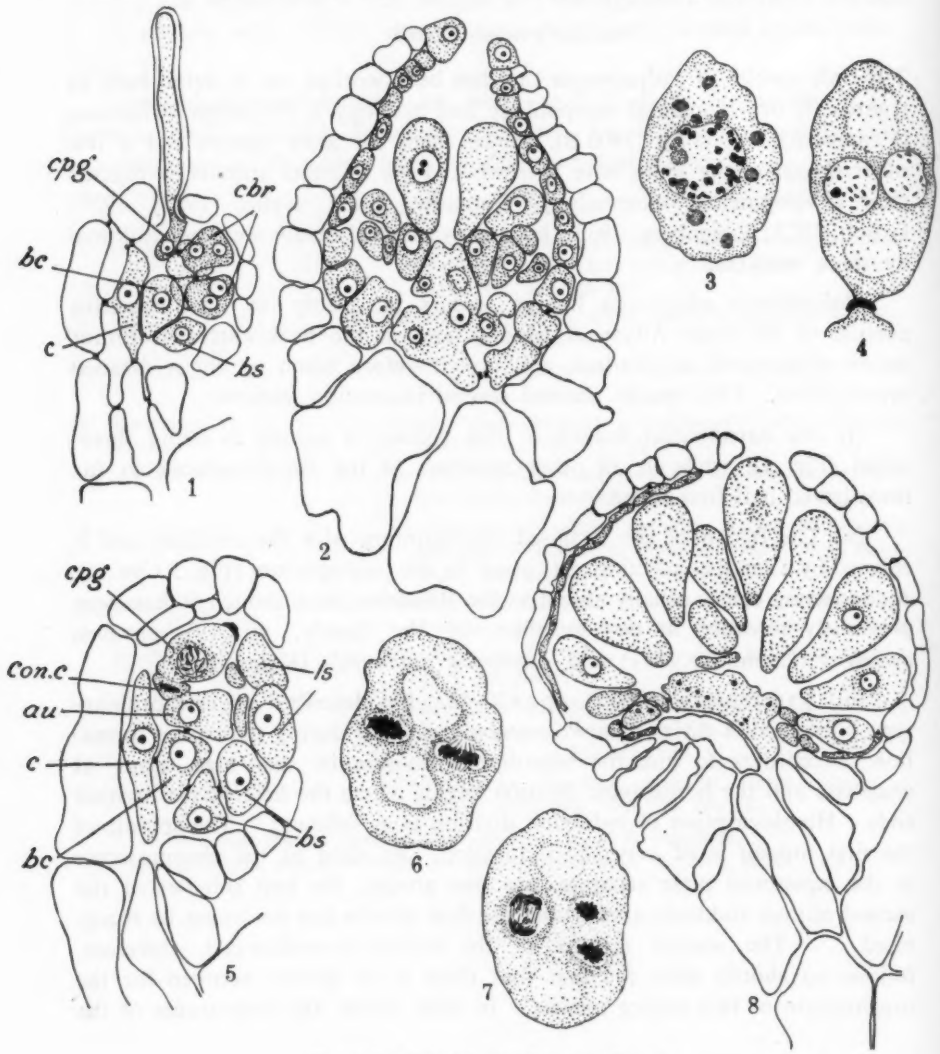
heterotypic division and the earlier stages of the homeotypic division are, as it were, completely dropped.

In the present form, however, no such extraordinary features were observed. The reduction division during tetraspore formation is quite normal. In the first division of the tetrasporangial nucleus, all the stages



Figs. 1–8. *Polysiphonia platycarpa* Boergs

Fig. 1. Procarp just before fertilisation, showing the bearing cell (*bc.*), basal sterile cell (*bs.*) and the three-celled carpogonial branch (*cbr.*); the two lateral sterile cells are shown behind the carpogonial branch. carpogonium (*cpg.*). ×900.

Fig. 2. Young cystocarp showing the two-layered cystocarp wall and its densely protoplasmic inner layer. ×600.

Fig. 3. Diakinesis in the tetrasporangium (only 20 pairs of chromosomes are seen in the section). ×1,125.

Fig. 4. Two-nucleate stage of the tetrasporangium. ×1,125.

Fig. 5. Section of procarp after fertilization, showing the auxiliary cell (*au.*), and the connecting cell (*con.c.*) between it and the carpogonium (*cpg.*). ×900.

Fig. 6. Metaphase of the second division in the tetrasporangium. ×1,125.

Fig. 7. Anaphase of the second division in the tetrasporangium. ×1,125.

Fig. 8. Ripe cystocarp with fully formed carpospores; note the wall is one-layered, with the remnants of the degenerating inner layer still sticking to it. ×600.

of the heterotypic division are gone through resulting in the formation of two daughter nuclei (Fig. 4). A definite interkinesis is seen after the first division before the commencement of the second division.

Again, according to Yamanouchi, the two spindles of the second division lie within the primary nuclear membrane which persists throughout the heterotypic and the homeotypic mitoses. In the present alga, unlike in *Polysiphonia violacea*, the two spindles of the homeotypic division lie separately in the tetrasporangium (Figs. 6, 7); the membrane of the tetrasporangial nucleus does not persist beyond the metaphase of the first division.

(iv) During the heterotypic mitosis, the tetrasporangial nucleus passes into an apparent resting stage after pachytene. Diakinesis (Fig. 3) is observed only after the tetrasporangium had enlarged considerably. During this enlargement, the tetrasporangial nucleus remains in an apparently resting condition. Such an intervention of an apparent resting stage between pachytene and diakinesis during reduction division is of very rare occurrence and has been recorded by Westbrook (1928, 1935) in *Rhodymenia palmata* and some other Florideæ. Similar resting stages during meiosis have been reported for some Phæophyceæ also, *viz.*, *Dictyota dichotoma* (Williams, 1904), *Padina pavonia* (Carter, 1927), and *Pylaiella littoralis* (Knight, 1923).

(v) The tetraspores appear to escape from the sporangium by squeezing themselves out through amæboid movement (Pl IX, Fig. 5).

(vi) The development of the cystocarp wall show certain interesting features. The cystocarp wall is two layered at the beginning (Fig. 2). Of these two layers, the outer layer is derived from the inner. During further development, the cells of the inner layer, which are rich in contents, gradually shrivel up and finally completely disappear, so that the wall of the mature

systocarp is made up of only one layer of cells, *viz.*, the original outer layer (Fig. 8). The inner layer is evidently nutritive in function.

Literature Cited

Carter, P. W. .. "The life-history of *Padina pavonia*. I. The structure and cytology of the tetrasporangial plant," *Ann. Bot.*, 1927 **41**, 139–59.

Connolly, C. J. .. "Beiträge zur Kenntnis einiger Florideen," *Flora*, 1911, **103**, 125–70.

Grubb, V. M. .. "The male organs of the Florideæ," *Journ. Linn. Soc. London, Bot.* 1925, **47**, 177–255.

Kylin, H. .. "Studien über die Entwicklungsgeschichte von *Rhodomela virgata* Kjellm.," *Svensk. Bot. Tidsskr.*, 1914, **8**, 33–69.

———— .. "Studien über die Entwicklungsgeschichte der Florideen," *Svensk. Vet. Akad. Handl.*, 1923, **63**, No. 11.

———— .. "Über Anatomie und Fruchtentwicklung bei *Polysiphonia urceolata*. "*Forhandl.*," *K. Fysiogr. Sällsk. Lund.*, 1937, **7**, No. 7.

Knight, M. .. "The life-history and cytology of *Pylaiella littoralis* Kjellm.," *Trans. Roy. Soc. Edinburgh*, 1923, **53**, 343–60.

Rosenberg, T. .. "Studien über Rhodomelaceen und Dasyaceen," *Akad. Abhandl. Lund.*, 1933.

Westbrook, M. A. .. "Contributions to the cytology of tetrasporic plants of *Rhodymenia palmata* (L.) Grev. and some other Florideæ," *Ann. Bot.*, 1928, **42**, 149–72.

———— .. "Observations on nuclear structure in the Florideæ," *Beih. Bot. Centralbl.* **A**, 1935, **53**, 564–85.

Williams, L. .. "Studies in the Dictyotaceæ. I. The cytology of the tetra-, sporangium and the germinating tetraspore," *Ann. Bot.*, 1904, **18**, 141–60.

Yamanouchi, S. .. "The life-history of *Polysiphonia violacea* (Preliminary note)," *Bot. Gaz.*, 1906 A, **41**, 425–33.

———— .. "The life-history of *Polysiphonia violacea*," *ibid.*, 1906 B, **42**, 401–49.

Explanation of Plates

Figs. 1–8. *Polysiphonia platycarpa* Boergs.

Fig. 1. Growing apex of a female plant showing the apical cell and a young procarp initial. ×330.

Fig. 2. Young procarp showing the three-celled carpogonial branch (*C*) and the bearing cell (*B.C.*). ×950.

Fig. 3. Apical portion of male plant showing antheridial clusters. ×75.

Fig. 4. Habit of alga growing epiphytically on leaf of *Halophila ovalis*. About natural size.

Fig. 5. Portions of tetrasporic plant with tetrasporangia. Note the escape of tetraspores in one of the sporangia. ×150.

Fig. 6. Part of a female plant with a young and an old cystocarp. ×160.

Fig. 7. Section of tetrasporangium showing the metaphase of the second division. ×1,450.

Fig. 8. Section of young cystocarp showing the developing carpospores and the two-layered wall. ×350.

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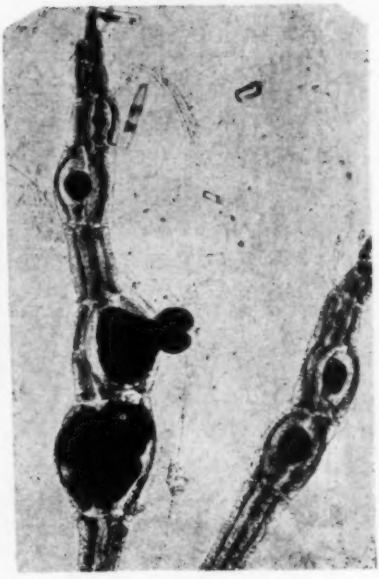

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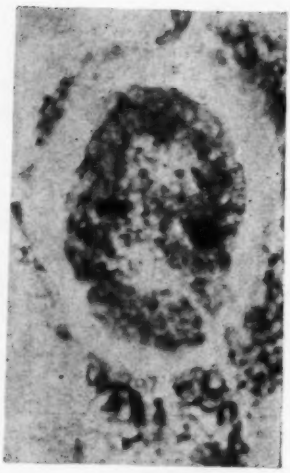

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8

STUDIES IN CROP PHYSIOLOGY

Nutrient Effects upon Development and Vegetative Vigour of Sugarcane

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Introduction

Recent nutritional studies on sugarcane have shown the overwhelming importance of nitrogen and phosphoric acid upon development and vegetative vigour of cane.[3] Specific deficiency and sufficiency symptoms of these and other ingredients have been worked out in an earlier contribution[5] alongside a systematic study of the general metabolic drifts at successive stages of the life-cycle.[4] The role of micro-nutrients such as boron has not been well worked out. Evidences regarding the role of this minor element and other major elements, in determining vegetative vigour of cane are presented in the following pages.

The investigations deal with three distinct aspects of sugarcane nutrition. In the first instance, an attempt is made to study the response of boron at different stages of sugarcane growth. Secondly, the relation between boron supply and deficiencies of nitrogen, phosphoric acid or potash, have been worked out under conditions of sand-fertiliser culture. Finally, an experiment has been planned to investigate the effects of boron as a fertiliser in soil; a factorial experiment with nitrogen, phosphoric acid and boron has been laid out to investigate the effects of these ingredients upon growth and development of cane at successive stages of life-cycle. Potash was avoided as no significant effect of this nutrient was recorded in these soils.

Experimentation

The three series of investigations formed a connected study on the nutrition of sugarcane. The experiments were conducted in 1945–46 on variety Co 312. The method of preparation of cultures was similar to that described earlier. Other details of experimental planning and procedure of work are given below.

Experiment I.—Boron effects upon development of cane grown in complete nutrient medium

Six concentrations of boron, *viz.*, 0, 5, 10, 15, 20 and 25 p.p.m. were applied as boric acid to November planted canes. Plants were grown in sand filled at the rate of 32 kgm. per cement concrete pot each 12 × 18 inches in size. Basal manuring of 150 p.p.m. of N, and 75 p.p.m. each of P and K, and a supply of one litre of Hoagland's complete nutrient solution containing all elements except boron, were given per culture to invigorate plants and enable them to grow under non-limiting conditions of these ingredients. Treatments were replicated five times, thus bringing the total number of cultures to 6 × 5 or 30. All the thirty cultures were kept under continuous observation for a period of over twelve months.

Experiment II.—Effects of boron supply and nutrient deficiencies upon growth and development of sugarcane

Six levels of boron nutrition, *viz.*, 0, 10, 20, 30, 40, 50 p.p.m. were supplied as boric acid in four instalments at 15 days interval to canes grown under four distinct conditions of mineral nutrition: (i) Complete nutrient culture (NPK), (ii) Nitrogen deficient culture (PK), (iii) Phosphorus deficient culture (NK) and (iv) Potash deficient culture (NP). The levels at which N, P and K were applied varied from 150 p.p.m. for N, to 75 p.p.m. for P and K. These were supplied in the form of ammonium sulphate, sodium phosphate, and sulphate of potash. Canes were planted in November 1944 in cement concrete pots 12 × 18 inches in size, each filled with 32 kgm. of sand. Treatments were replicated five times thus bringing the total number of cultures to 4 × 6 × 5 or 120. Hoagland's micronutrient solution minus boron was also applied at one litre per pot early in the life-cycle.

Experiment III.—Effects of nitrogen, phosphoric acid and boron on vegetative vigour, and juice quality of cane grown in soil

A 2 × 2 × 2 factorial experiment was laid out under the conditions of pot culture, where sugarcane was grown in soil supplied with ammonium sulphate, sodium phosphate and borax at 0 and 150 p.p.m. of N, 0 and 75 p.p.m. of P, and 0 and 5 p.p.m. of boron. In all eight combinations of these ingredients were applied to each pot filled with 27 kgm. of sandy loam soil. Watering and hoeing were done when needed. Treatments were replicated three times. Five pots per treatment were maintained thus bringing the total number of cultures to 5 × 3 × 8 or 120. Canes were planted in November 1944 and harvested early in January 1946.

Characters studied.—Growth studies involved measurement of height of main shoot, number of tillers and number of green and dry leaves at nine successive monthly stages between the age of 30 and 270 days. Weights of roots, stripped canes, and leaves, along side an estimation of Sucrose %, °Brix, Glucose % and Purity Coefficient, were also determined at harvest. The procedure of recording the vegetative characters and of estimating the juice quality was similar to that adopted in earlier investigation.[4] Summarised results of these findings are indicated in various text tables and figures.

Experimental Results

Experiment I.—Statistical analysis of the data showed that the effect of age was significant on all developmental characters; boron effects were however, significant in case of height, tillers and dry leaves only (Table I). All doses higher than 5 p.p.m. (excepting 20 p.p.m.) significantly increased height (Table II) over that of control. The first three successive levels of 5, 10 and 15 p.p.m. showed positive effects but the increases over the respective lower levels were not significant at all. Marked reductions were noted in cultures where B was raised from 15 to 20 p.p.m. Further increase to 25 p.p.m. again raised height over that recorded for either 20 p.p.m. or the control. Age had no significant effect upto the fourth month (Table III). Between 4–8 months significant increases in height due to advancing age were obtained. Towards ninth month again age-effects were insignificant.

Table I

Height of main shoot in sugarcane Co 312

Analysis of variance

Due to	Degrees of Freedom	Mean sum of squares			
		Height	Tillers	Green leaf	Dry leaves
Replications ..	1	102	4	3·0*	5·0*
Boron ..	5	460*	10·2*	1·6	14·8*
Age ..	8	28141*	252*	22·1*	970·7*
Boron × Age ..	40	70·5	4·2	3·2*	1·9
Error ..	53	83·3	6·2	1·7	1·7
Total ..	107	28856·8	276·6	31·6	994·1
S. E. per pot ..	..	9·1	2·49	1·3	1·3

* Significant effects

TABLE II

Over-all-age effects of boron upon plant characters

Characters	Concentration of B in p.p.m.										S. E. Difference
	0		5		10		15		20		25	
Height cm. ..	53		54		59·5		60·5		53·5		65·0	
Increase ..		*1·0*		*5·5*		*1·0*		*-7·0*		*12·0*		*2·97*
Tillers ..	7·1		8·1		8·7		7·4		9·0		8·3	
Increase ..		*1·0*		*0·6*		*-1·3*		*1·6*		*-0·7*		*0·79*
Green leaves..	7·0		6·8		7·2		7·1		6·6		7·5	
Increase ..		*-0·2*		*0·4*		*-0·1*		*-0·5*		*+0·9*		*0·43*
Dry leaves ..	14·7		13·5		22·0		12·6		14·6		15·0	
Increase ..		*-1·2*		*8·6*		*-9·4*		*2·0*		*0·4*		*0·43*

TABLE III

Over-all-boron effects of age upon plant characters

Character	Months after planting																		S. E. of difference
	1		2		3		4		5		6		7		8		9		
Height cm...	10·0		16·1		19·3		21·8		33·3		68·8		94·6		124·6		131·5		
Increases		*6·1*		*3·2*		*2·5*		*12·5*		*35·5*		*25·8*		*29·9*		*7·0*		*3·7*
Tillers ..	3·8		7·6		12·6		16·7		11·8		6·6		5·4		4·6		3·7		
Increases		*3·8*		*5·0*		*4·1*		*-5·1*		*-5·2*		*-1·2*		*-0·8*		*-0·9*		*0·97*
Green leaves	7·8		8·5		6·9		7·05		5·88		7·6		7·5		6·4		4·05		
Increases		*0·7*		*-1·6*		*0·1*		*-1·2*		*1·8*		*-0·1*		*-1·1*		*-2·4*		*0·52*
Dry leaves ..	2·4		5·1		7·9		9·3		14·0		16·2		18·9		24·3		29·4		
Increases		*2·7*		*2·8*		*1·4*		*5·3*		*2·2*		*2·7*		*5·4*		*8·1*		*0·52*

Tillers per plant were again significantly affected by age (Table I). Increased supply of boron at 10 and 20 p.p.m. increased tillering significantly over that of the control (Table II). Of all successive levels, increases from 15–20 p.p.m. only augmented tillering significantly.

Age raised number of tillers during the first four months of the life-cycle (Table III). At later stages the number of shoots fell down. Increases in tillering between the 1–2, and 2–3 months and decreases between 4–5, and 5–6 months were highly significant.

Green leaves.—On number of green leaves boron effects were insignificant (Table I); this was true for the majority of the levels of this ingredient. Successive levels upto 20 p.p.m. were equally insignificant in effect (Table II). Between 20–25 p.p.m. only, boron raised the number of green leaves. A

significant reduction in green leaves on the main shoot was evidenced particularly between 2–3, 4–5, 7–8 and 8–9 months of the life-cycle. Between 5–6 months, on the contrary, leaf number was significantly raised.

Dry leaves.—On number of dry leaves on the main shoot, both boron and age showed significant effects (Table I). Boron at 10 p.p.m. appeared to cause maximum drying of leaves; plants under this showed greater dry leaf number both in comparison to control and other levels of boron (Table II). Increases from 5–10 p.p.m. or from 15–20 p.p.m. were positively significant. Number of dry leaves also increased with advance in age of cane (Table III).

Characters at harvest.—Increase in boron supply also showed some useful effects on the yield of cane (Table IV). 20 p.p.m. B uniformly showed good effects on weight of cane, roots and leaves. On juice characters 25 p.p.m. was deleterious. 15 p.p.m. and 5 p.p.m. were better for sucrose and °Brix. Purity coefficient under 15 and 20 p.p.m. was slightly better than other levels but in none of these cases, was the purity better than the control.

TABLE IV

Boron effects upon plant and juice characters at harvest

Characters	Concentration of B in p.p.m.					
	0	5	10	15	20	25
Wt. of cane oz.	95	97	97·5	89·0	99·0	92·0
Wt. of leaf oz.	18·5	17·0	17·0	14·0	18·5	14·0
Wt. of root oz.	17·5	14·5	21·5	24·0	26·5	22·5
Entire plant ..	130·0	128·5	136·0	127·0	144·0	128·5
°Brix in juice ..	18·6	21·2	19·4	20·2	19·4	14·2
Sucrose % ..	16·28	16·62	15·65	17·25	16·48	9·4
Glucose % ..	1·57	0·96	0·51	0·42	0·75	0·4
Purity coef. ..	87·5	78·5	80·7	86·4	85·0	66·5

EXPERIMENT II.—Analysis of data showed that the main effects of age, boron, and nutrient deficiencies were highly significant on height of cane. (Table V). First order interaction between fertilisers deficiency × age, and higher order interactions between fertiliser deficiency × age × boron were also highly significant. Advance in age significantly raised height of main shoot throughout the major portion of the life-cycle (Table VI). Phosphorus deficiency lowered this character to the maximum; next in order were nitrogen and potash-deficient plants (Table VII). Complete nutrient cultures produced the tallest canes. Differences in height in – K and – P and between – P and – N were statistically significant. Boron showed

TABLE V

Plant characters of sugarcane Co 312 Expt. II

Analysis of variance

Due to	Degree of freedom	Mean sum of squares			
		Height	Tillers	Green leaf	Dry leaf
Replication ..	1	817**	667**	7·0**	54**
Age ..	8	100812**	605**	31·0**	2257**
Boron ..	5	236**	35**	00·8	14·6**
Fertiliser def. ..	3	7103**	325**	11·6*	66·3**
Boron × age ..	40	55	4·6**	2·4**	2·6
Fert. def. × Age ..	24	388**	33·2**	8·0**	5·7*
Fert. def. × Boron ..	15	63	15·4**	28·0	34·7**
Fert. def. × Age × Boron ..	120	381**	2·8**	0·0	2·3
Error ..	215	37	0·32	0·7	3·8
Total ..	431	109892	1688·32	89·5	2441·0
S.E./observation ..		6·09	0·56	0·83	1·96

TABLE VI

Over-all-fertiliser effects of age upon plant characters

Characters	Age in months after in planting																		S. E. of difference
	10		2		3		4		5		6		7		8		9		
Height ..	14·5		18·4		20·0		24·1		35·3		71·6		91·4		117·2		131·2		
Increases		3·9		1·6		4·1		11·2		36·3		19·8		26·3		13·5			1·2
Tillers ..	1·5		7·7		9·9		11·2		6·1		3·5		3·1		2·6		2·1		
Increases		6·2		2·2		1·3		−5·1		−2·6		0·4		0·5		−0·5			0·11
Green leaves	6·8		9·0		8·0		7·8		8·7		8·8		7·9		8·0		6·7		
Increases		2·2		−1·0		−0·2		0·9		0·1		−0·9		0·1		−1·3			0·32
Dry leaves ..	3·9		5·1		7·5		9·3		12·5		14·4		15·9		20·8		23·8		
Increases		1·2		2·4		1·8		3·2		1·9		1·5		4·9		3·0			0·39

TABLE VII

Over-all-age effects of mineral deficiencies upon plant characters

Characters	Condition of nutrition								S.E. of difference
	F.M.		−K		−P		−N		
Height ..	67·8		61·3		49·1		54·9		
Increases ..		−6·5		−12·2		5·8			0·82
Tillers ..	6·8		6·5		4·5		3·4		
Increases ..		−0·3		−2·0		−1·1			0·07
Green leaves ..	8·4		7·7		7·7		8·2		
Increases ..		−0·7		0·0		0·5			0·21
Dry leaves ..	12·4		13·1		11·7		13·2		
Increases ..		0·7		−1·4		1·5			0·25

some augmentative effects upto 10 p.p.m. (Table VIII). Canes under this treatment were significantly superior to those under other doses of boron as well.

Tillering.—All main effects and interactions showed significant effects on tillering (Table V). Number of tillers was increased upto four months of the life-cycle after which advance in age reduced this character (Table VI). Most of the increases during early stages and decreases during later part of the life-cycle were found to be statistically significant. Deficiency of nutrients lowered tillering; maximum reduction took place in — N, next in order were — P and — K plants. Differences in tillers between complete nutrient and — K, between — K and — P, and between — P and — N were found to be statistically significant. Boron upto 10 p.p.m. produced largest number of tillers; higher concentrations lowered it. The increases or decreases in most cases were significant (Table VIII).

TABLE VIII

Over-all-age effects of boron upon plant characters

Characters	Concentration of B in p.p.m.										S. E. of Difference
	0		10		20		30		40		50	
Height ..	58·1		60·5		58·5		59·6		55·3		57·4	
Increases		*2·4*		*-2·0*		*1·1*		*-4·3*		*2·1*		*1·00*
Tillers ..	5·9		6·1		5·8		4·7		5·0		4·4	
Increases		*0·2*		*-0·3*		*-1·1*		*0·3*		*-0·6*		*0·08*
Green leaves	8·0		7·8		8·0		8·2		8·0		8·0	
Increases		*-0·2*		*-0·2*		*-0·2*		*-0·2*		*0·0*		*0·25*
Dry Leaves	12·7		13·1		13·0		12·1		12·0		12·6	
Increases		*0·4*		*0·1*		*0·9*		*-0·1*		*0·6*		*0·32*

Green leaves.—Age and nutrient deficiencies significantly affected green leaves (Table V); boron effects, on the other hand, were not significant. Interactions between boron and age, between fertiliser deficiency and age, and between fertiliser deficiency and boron, were also significant. Age increased leaf number on the main shoot upto 60 days only (Table VI); at later stages it reduced green leaf number on main shoot. Secondary rise in green leaf number of a lesser magnitude was noted between 150–180 days. Increases observed between the ages of 30 and 60 days, and between 120 and 150 days were significant. Significant reductions were also recorded between 60–90 days, between 180–210, and between 240–270 days in the life-cycle. Complete nutrient plants were better than — K and — P plants (Table VII); differences between — N and complete nutrient plants were insignificant. Boron concentration did not markedly affect this character though 30 p.p.m. proved slightly better than other concentrations (Table VIII).

Dry leaves.—Age, boron and fertiliser deficiencies—all significantly affected number of dry leaves. Interaction between fertiliser deficiency and boron and between fertiliser deficiency and age, were also significant (Table V). Age significantly increased dry leaf number (Table VI); their number in – K and – N plants was greater than in full manure or – P plants. Differences between the full nutrient and – K, between – K and – P, and between – P and – N were highly significant (Table VII). Increase in concentration of boron upto 10 p.p.m. showed tendency to raise number of dry leaves while higher doses reduced it (Table VIII).

Characters at harvest.—Sucrose percentage at harvest was maximum in cultures supplied with 30 p.p.m. of boron. Subsequent increases lowered this (Table IX). Addition of boron had little effect in potash deficient cultures. In – P medium 10–30 p.p.m. of boron raised sucrose. Highest values were obtained at 50 p.p.m. in – P cultures and 40 p.p.m. in – N cultures. °Brix was improved by boron application at 20–30 p.p.m. in complete nutrient culture, 20 p.p.m. in potash deficient culture, 0–10 p.p.m. in – N cultures, and 50 p.p.m. in – P medium. Percentage of glucose was also affected by boron. In – P plants addition of this ingredient reduced glucose percentage; in – N, the effects of similar doses were not at all marked. In complete nutrient cultures boron addition raised glucose to a high value within the concentration range of 20–50 p.p.m. Purity of cane juice in complete nutrient and – K medium was less affected by boron; in – N or – P medium this micro-element showed better effects. In the latter case as high as 50 p.p.m proved better than other ranges of this nutrient. Irrespective of the state of mineral deficiency, boron showed a tendency to increase weight of plant at harvest. Relatively higher yields of stripped cane, were recorded at 10 and 30 p.p.m. Weight of leaves was higher under 0 and 20 p.p.m. Higher levels of the order of 30 p.p.m. proved better from the point of view of root weight and weight of entire plant (Table IX).

TABLE IX

Effect of boron upon growth characters at harvest

Nutrient medium	Dose of boron in p.p.m.					
	0	10	20	30	40	50
	1. *Wt. of stripped cane in oz./2 pots*					
Complete Sol. ..	93	75	96	83	82	96
– K ..	59	85	84	83	85	79
– P ..	60	53	42	49	42	40
– N ..	28	50	28	47	31	25
Mean ..	60	66	62	65	60	60

TABLE IX—(*Contd.*)

Nutrition	Dose of born in p.p.m.					
	0	10	20	30	40	50
	2. *Weight of green and dry leaves at harvest (oz./2 pots)*					
Complete Sol. ..	12	10·5	16	11	10·5	13
– K ..	10·5	10	11	10·5	11	10·5
– P ..	10·5	7·5	6	6	5·5	5·5
– N ..	3·5	7·5	3·5	4·5	4·5	3·5
Mean ..	12·2	11·8	12·2	8·0	7·75	8·1
	3. *Root wt. in oz./2 pots at harvest*					
Complete Sol. ..	17·5	22·0	20·0	29·0	20·5	22·0
– K ..	11·5	18·5	18·5	14·5	20·5	20·5
– P ..	10·5	15·5	14·5	13·5	16·5	12·5
– N ..	8·5	11·5	7·5	17·0	9·0	5·5
Mean ..	12·0	16·9	15·1	18·5	16·6	15·1
	4. *Total wt. in oz./2 pots at harvest*					
Complete Sol. ..	122	107	132	123	113	131
– K ..	81	108	118	108	116	110
– P ..	81	76	62	69	64	58
– N ..	40	69	39	68	44	34
Mean ..	81	90	88	92	84	83
	5. *°Brix of cane juice at harvest*					
Complete Sol. ..	21·0	20·4	21·4	21·4	20·4	20·6
– K ..	20·4	22·2	22·6	21·4	22·2	21·2
– P ..	20·6	22·0	22·2	22·4	21·6	21·2
– N ..	22·4	22·0	21·0	22·0	22·0	20·6
Mean ..	21·1	21·6	21·8	21·8	21·5	21·4
	6. *Sucrose % in cane juice at harvest*					
Complete Sol. ..	18·3	17·5	16·7	18·2	17·6	17·3
– K ..	15·4	19·2	19·4	18·4	19·3	18·4
– P ..	17·1	19·2	19·9	20·2	19·6	21·6
– N ..	20·1	19·6	18·6	19·7	19·8	18·4
Mean ..	17·7	18·9	18·6	19·1	19·0	18·9
	7. *Glucose % in cane juice at harvest*					
Complete Sol. ..	0·43	0·73	0·24	0·42	0·57	0·70
– K ..	1·03	0·54	0·57	0·92	0·24	0·43
– P ..	0·52	0·36	0·19	0·20	0·16	0·13
– N ..	0·18	0·16	0·21	0·15	0·15	0·16
Mean ..	0·54	0·49	0·30	0·42	0·28	0·36
	8. *Purity coefficient of cane juice at harvest*					
Complete Sol. ..	87·1	85·6	87·2	84·8	86·2	83·8
– K ..	75·5	86·2	85·7	82·0	86·8	86·7
– P ..	83·0	87·2	89·7	90·2	90·6	93·1
– N ..	89·6	89·2	88·5	89·3	90·2	89·3
Mean ..	83·8	87·0	87·8	86·6	88·4	88·2

EXPERIMENT III.—Data collected on the effects of N, P and B in these soils, showed that height of main shoot varied considerably in response to both age and fertilisers (Table X). Significant effects of N were only recorded at 60 and 180 days and of P at 30, 210 and 270 days; the responses

TABLE X

Nutrient effects upon plant characters at successive stages

Age.	Characters	N	P	B	NP	NB	PB	NPB	S. E. of main effects and Int.
30	Height ..	3·0	13·0**	− 9·0	−12·6*	− 9·8	16·2	− 0·2	5·25
	Tillers ..	13·2*	0·8	− 6·8	− 5·6	0·0	0·2	1·2	5·56
	Green leaf .	−0·2	3·6	−12·4**	− 1·8	− 5·4	12·0	2·6	3·52
	Dry leaf ..	4·3*	1·3	6·1*	− 0·9	3·1	− 2·3	3·5	2·07
60	Height ..	23·7*	−1·4	3·4	−28·2**	−21·8*	20·2*	13·4	9·28
	Tillers .	7·5	−5·5	5·5	− 1·5	− 6·5	8·5	14·5**	4·69
	Green leaf ..	−10·5	−1·5	−10·5	6·5	− 4·5	6·5	−15·5	8·58
	Dry leaf ..	8·5	- 0·5	15·5*	− 9·5	6·5	9·5	12·5	6·73
90	Height ..	24·5	11·1	13·1	0·3	−36·9**	− 7·1	14·1	12·32
	Tillers ..	35·5**	−7·5	14·5	3·5	13·5	3·5	7·5	9·16
	Green leaf ..	9·0*	9·0*	3·0	7·0	−11·0*	−15·0**	− 1·0	4·24
	Dry leaf ..	1·5	−9·5	−0·5	− 3·5	2·5	20·5**	3·5	5·62
120	Height .	42·0	12·8	18·0	−10·4	−82·4*	10·6	21·0	32·96
	Tillers ..	83·0**	9·0	29·0**	5·0	19·0*	− 7·0	1·0	9·45
	Green leaf ..	−4·0	1·5	3·5	3·0	− 5·0	− 2·0	6·0	9·88
	Dry leaf ..	8·5	−5·5	−0·5	0·5	- 15·5*	7·5	1·5	5·04
150	Height ..	−16·9	53·3	52·4	−21·3	−77·9*	− 17·7	16·9	32·6
	Tillers ..	77·9**	5·0	11·3	− 3·0	5·0	3·0	3·0	8·3
	Green leaf ..	− 20·0	2·0	7·0	0·0	1·0	−36·0*	− 4·0	13·5
	Dry leaf ..	11·0	−5·0	−13·0	4·0	−19·0*	9·0**	9·0	3·0
180	Height ..	22·5*	10·5	−1·5	9·5	−16·5**	−34·0**	34·5**	10·8
	Tillers ..	39·0**	6·5	5·0	−4·0	1·0	2·0	−0·5	6·3
	Green leaf ..	− 16·5*	4·5	−5·5	−0·5	− 9·5	−8·5	−4·5	8·3
	Dry leaf ..	12·5*	−5·0	−0·5	0·5	−28·5	8·5	8·5	6·2
210	Height ..	− 37·0	123·0**	11·0	−83·0**	−41·0	−75·0*	9·0	27·8
	Tillers ..	29·5**	11·5**	10·0**	−0·5	6·5	6·5	6·5	3·9
	Green leaf ..	−15·5**	1·5	−0·5	− 2·5	− 2·5	−7·5	−1·5	4·8
	Dry leaf ..	9·5	−18·5*	36·0**	2·5	−27·5**	5·5	7·5	8·3
240	Height ..	34·0	96·0	8·0	−58·0	−89·0	−114·0	68·0	70·8
	Tillers ..	28·0**	5·0	13·5**	− 2·0	10·6	6·0	6·0	5·3
	Green leaf ..	− 9·5	7·0	−0·5	1·5	−5·0	−5·0	3·0	5·01
	Dry leaf ..	9·0	9·5	11·5	0·5	−84·5*	−0·5	8·0	9·1
270	Height ..	9·0	149·0**	51·0	−117·0**	−127·0**	−131·0**	35·0	34·9
	Tillers ..	23·0**	2·5	7·0	− 3·0	3·5	5·0	10·0	5·4
	Green leaf ..	−36·5**	14·5*	6·5	− 3·5	− 3·5	−12·5	− 4·5	7·06
	Dry leaf ..	42·0**	− 4·0	8·0	− 2·0	−24·0	2·0	16·0	11·5

in both these cases were positive. Boron did not exhibit any significant effect on height. Nitrogen showed significant increases on tillering at all stages throughout the life-cycle. P and B also tended to increase this character but the effects were not as conspicuous as that of nitrogen; significant effects were only recorded towards the later stages of growth (210 days). Formation of green leaves was checked by boron during the first month of its application; no significant effects were noted at later periods. N on the contrary, improved formation of green leaves during early stages of 90 days but at later periods of the life-cycle (180, 210, 270 days) significantly depressed this character. P showed positively significant effects at 90 and 270 days only. Number of dry leaves was increased both by nitrogen and boron application at 30 days and by B alone at 30 and 60 days of early growth of cane. Interactions N × P and N × B showed negatively significant response, if at all, on height and green and dry leaves on main shoot. The latter (N × B) showed positively significant effects on tillering at 120 and 240 days of the life cycle only. P × B interaction gave positively significant effect on height at 30 and 60 days and negative effects at 180, 210 and 270 days. Higher order interactions showed positive effects on height at 180 days and on tillers at 60 days period of observation.

Nitrogen in combination with P improved weight of stripped cane and weight of entire plant; with both P and B, it increased °Brix. Phosphorus alone helped to improve sucrose and purity of cane. Boron in combination with P was most helpful from the point of view of root weight only (Table XI).

TABLE XI

Growth characters at harvest

Characters	O	N	P	B	NP	NB	PB	NPB
Cane wt. oz. ..	119	82	29	37	161	113	83	127
Leaf wt. oz. ..	21	31	17	14	30	22	20	31
Root wt. oz. ..	60	82	70	46	69	25	99	46
Whole plant ..	200	195	116	97	260	160	202	204
°Brix .	18·8	14·0	19·2	19·4	14·0	18·4	20·2	21·4
Sucrose % .	16·28	9·86	17·41	16·77	9·96	14·41	16·89	15·19
Glucose % ..	0·39	2·21	0·95	0·44	2·40	0·69	0·43	0·23
Purity coef. ..	86·6	70·4	90·6	86·4	71·1	78·3	83·6	70·2

DISCUSSION

A. *Nutrient effects upon vegetative vigour*

Data collected on the effects of macro and micro nutrients upon growth and development of sugarcane point out the importance of these ingredients

on several plant characters. While the general tendency of the application of nutrients to soil has been to promote development of the sugarcane plant, the effects produced were prominently significant in some directions only. Thus nitrogen exhibited the most significant effect in increasing tillering at almost all periods of the life-cycle (Table X). Phosphoric acid, on the contrary, was more useful in increasing height of shoot; its best effects were noticeable towards the later stages of the life-cycle. Nitrogen also differed from phosphoric acid in the positively significant effects produced on the number of dry leaves; this was at least noticeable at four stages of growth. Green leaves were reduced towards later periods when the plant in general exhibited a tendency to rapid desiccation and drying of leaves. How was this desiccation brought about in spite of favourable soil moisture conditions cannot be said with certainty for the present. Evidence collected, however, point out that the nitrogen fed canes as a rule under the conditions of the experiment exhibited a general tendency to develop tillers in larger numbers. This development of large number of shoots required the movement of larger quantity of nutrients and raw materials towards the tillers more than to the main shoot. Drying of leaves on main shoot thus appeared in all probability to be a physiological effect of limited nutrient and water supply of the main shoot caused by excessive tillering in response to nitrogen feeding. As against these major ingredients boron was distinct in effect in so far as it did not exhibit any significant responses on height at any stage of the life-cycle; its effect on tillering was useful markedly at three stages. A general tendency to poor development of leaves and to their rapid drying was also noticeable during early stages of the life-cycle in Experiment 3.

That boron supply was most helpful in at least partially overcoming the deficiency effects of certain major elements is well borne out by the results obtained in Experiment II. This is most apparent in case of yield and juice characters. While none of the deficient cultures attained the level of complete nutrient cultures, only $-K$ plants when supplemented with adequate amounts of this ingredient produced more or less the same yield as the fully manured canes under similar conditions of boron supply (Fig. 1). Reduction in yield caused by potash deficiency could thus be partly made up by small doses of boron added to the medium of growth. The effect of this ingredient was all the more clear in case of juice characters. Thus in presence of boron, even $-P$ and $-K$ canes maintained fairly high sucrose content and purity of juice; in the absence of this micro-element, lack of these two elements, however, lowered sucrose and purity markedly. Boron thus helps in improving juice quality. It may to a certain extent work as a substitute for P and K and may help to overcome conditions of partial

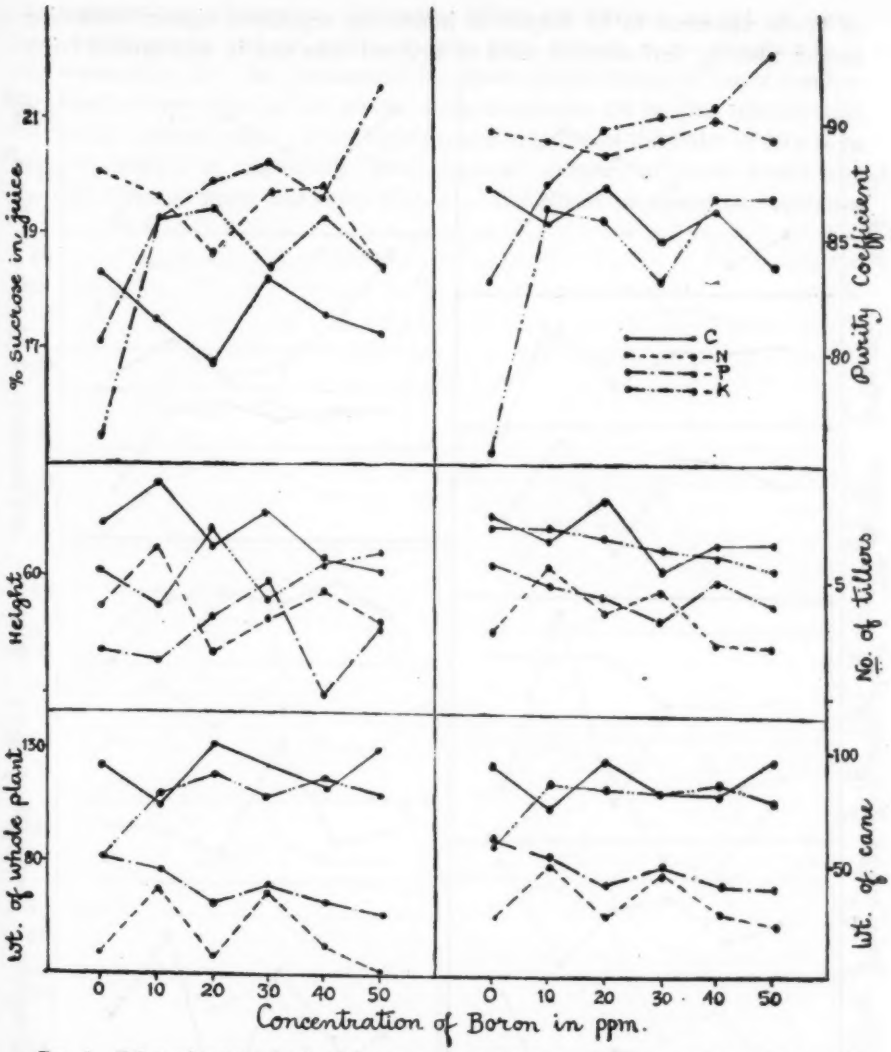

FIG. 1. Effect of varying levels of boron under different conditions of nutrient deficiency

deficiency of these ingredients under conditions of the field. The exact mechanism of response however remains yet to be worked out.

The level at which boron is most helpful has been found to vary with the plant character, the conditions of the experiment and more particularly the state of deficiency of major ingredients. Thus in Experiment I, 10 p.p.m.

of boron appeared to be helpful in improving vegetative vigour, increasing height, tillering, leaf number, yield of stripped cane and in maintaining fairly

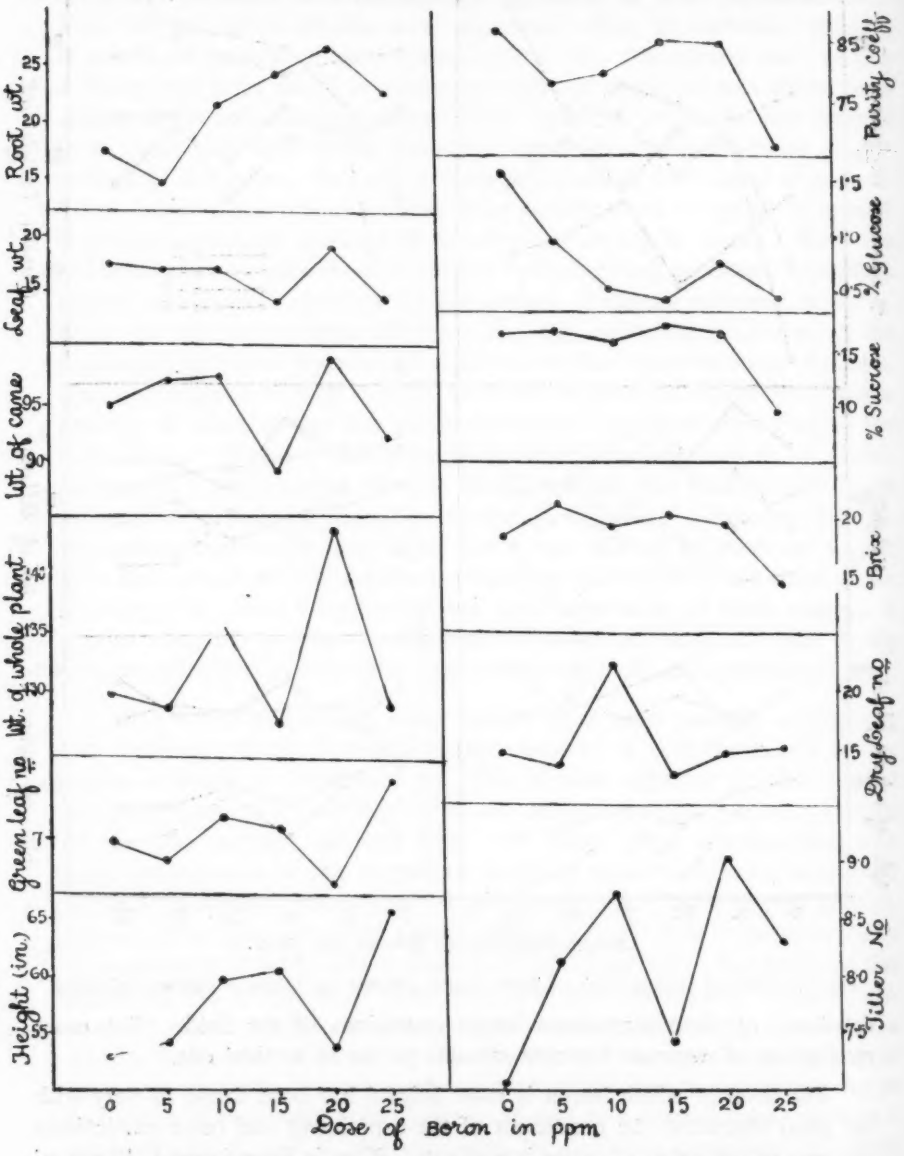

FIG. 2. Effect of varying concentrations of boron upon plant characters, (Expt. I)

high percentage of sucrose. Its useful effects on tillering, weight of roots, and entire plants and on purity of juice were evident even upto 20 p.p.m. of boron (Fig. 2). In Experiment II, again useful effects of lower concentrations of the order of 10 p.p.m. were noticeable on height, tillering and weight of stripped cane. Even higher concentrations of the order of 30 p.p.m. proved helpful in improving °Brix, sucrose, number of green leaves and weight of entire plant and roots (Fig. 3). The effects produced on vegetative

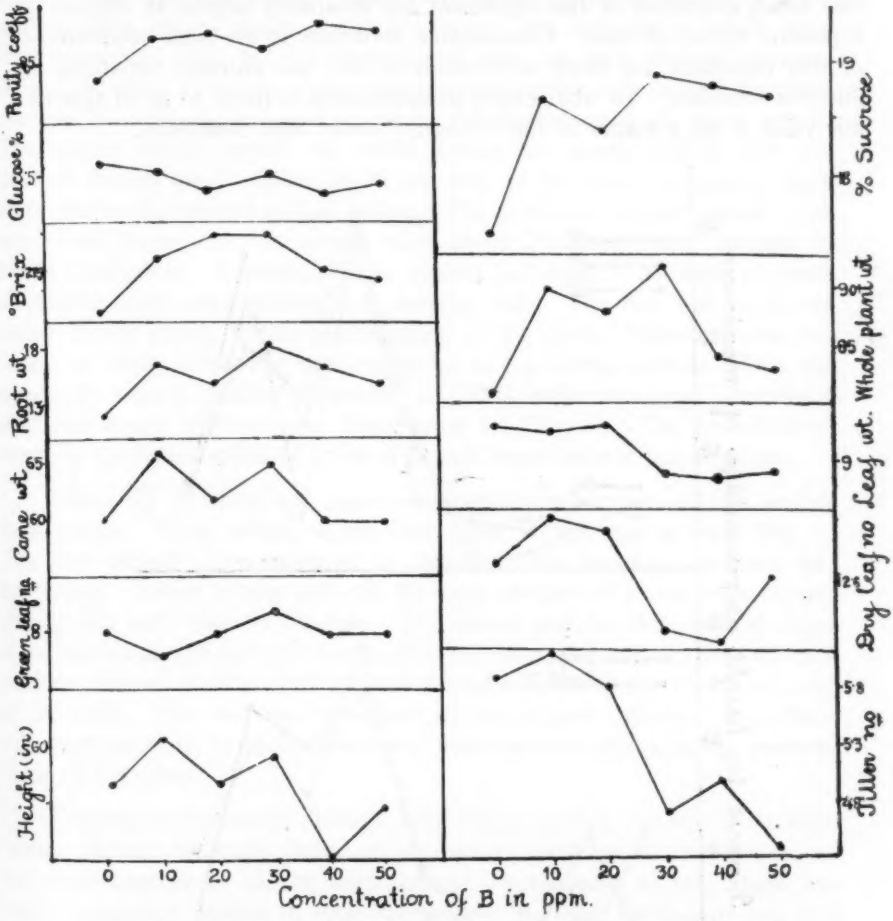

FIG. 3. Effects of varying doses of boron upon plant characters (Expt. II)

vigour appear primarily to be due to better development of roots in presence of this micro-element and secondly due to improvement in the general physiological tone of the plant as a whole.

The results give overwhelming support to the view that boron is helpful to sugarcane plant in a variety of ways when applied in suitable doses. The concentrations found useful have been noted to vary considerably depending upon the general nutrient status of soil. Under conditions of phosphorus and potash deficiencies, it improves juice quality while under the latter, it helps in improving vegetative vigour and yield of plant. The results are in agreement with the findings of other investigators [1, 2] who have shown that small quantities of this ingredient are invariably helpful in improving vegetative vigour of cane. Considerable variations in the basic requirement of this ingredient are likely as a result of soil and climatic variations in different localities. To what extent its application is likely to be of agronomic value is yet a matter of future enquiry under field conditions.

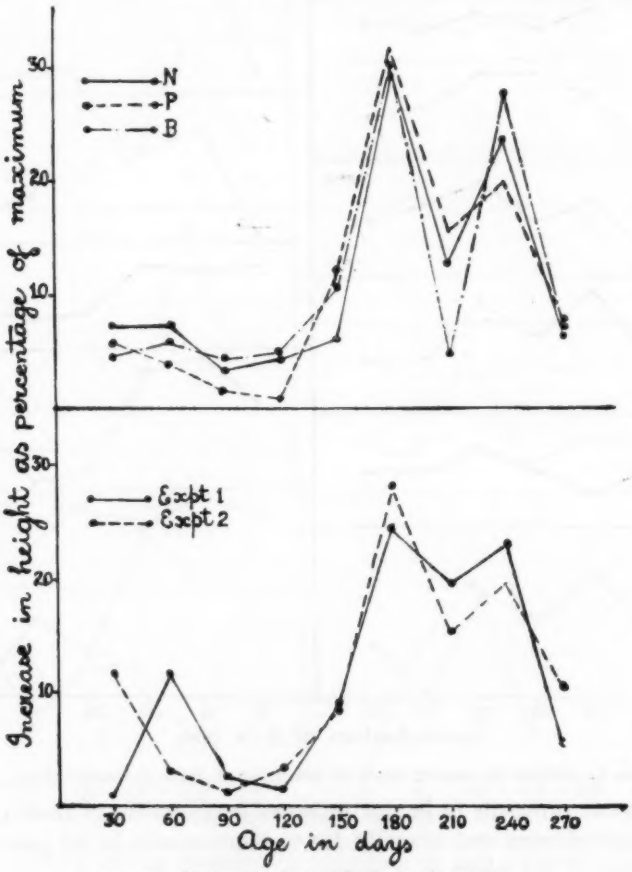

FIG. 4. Critical periods of shoot elongation

B. *Critical periods of plant development*

Coming now to the question of development of the plant, the increases or decreases in height, tillering and leaf number during successive intervals of the life-cycle have been calculated as percentage of recorded maximum of these characters in various experiments. This has been done to indicate the contributions made by each interval of the life cycle towards the development of the sugarcane plant. Calculating in this way, the sugarcane plant showed three critical stages in the life cycle when increases in height were most prominent (Fig. 4). The first critical stage has been recorded between 30 and 60 days in all the three experiments; during this period the plant contributed only 10 per cent. towards the development of shoot length. The second critical period was noted during the month ending with 180 days of the life cycle; about 25–30 per cent. of the shoot elongation took place during this second critical period. The third and the last critical stage was noted during the 8th month when about 20–25 per cent. increase in height took place. It would thus be evident that over 50 per cent. of shoot elongation took place during 6–8 months only. The rest was made up rather slowly mostly during earlier stages of life cycle. Nutrients were not found to bring about any marked change in the critical periods. This was shown by almost similar behaviour of plants under nitrogen, phosphoric acid and boron treatments in Experiment III (Fig. 4). The contributions made by age factor appeared to be of greater importance in this direction.

Formation of leaves was more prominent during certain specific periods than others. Three critical stages were noted in this case as well (Fig. 5). The first critical stage appeared to contribute the maximum towards leaf formation. About 30 per cent. of the total number of leaves were formed during this early stage of 30 days. The second and the third critical stages were located at 150 and 240 days in the life cycle; the contributions of each of these periods towards leaf formation amounted to about 10–15 per cent. of the total. This was true for almost all the nutrient cultures. Age effects appeared again to be more prominent than nutrient effects in the development of the plant.

Tillering in sugarcane showed again three critical periods of development. During the early period of 60 days as much as 40–50 per cent. of the total number of shoots were formed. Subsequent to this, there was noted a marked decline in tiller formation. Between the fourth and fifth month (the second critical stage), mortality of tillers was noted. Maximum mortality was noted in the fifth month. A decline in rate of mortality soon ensued; a sort of recovery was noted between 7–9 months. This in a way

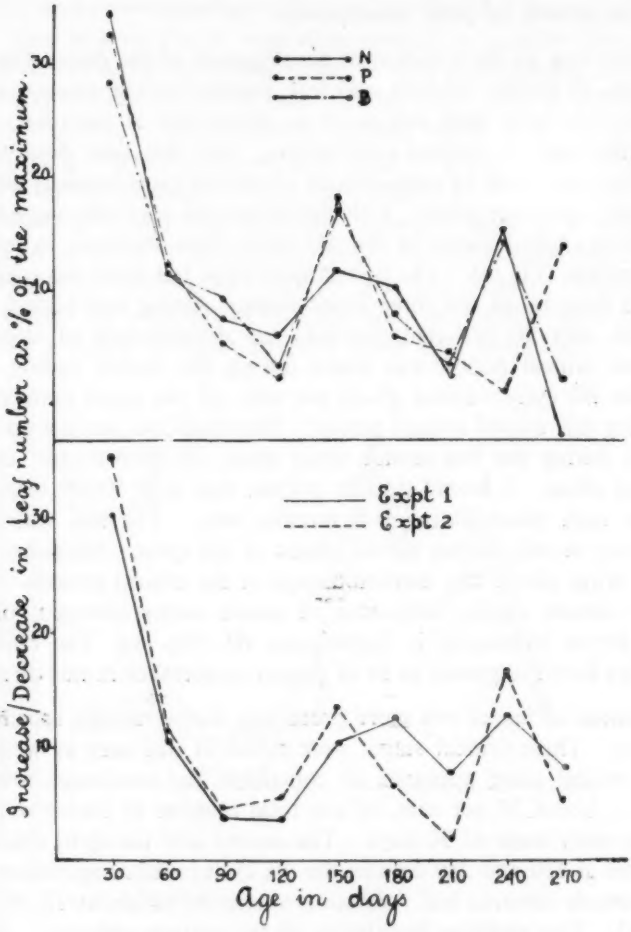

FIG. 5. Critical periods of leaf development

constituted the third critical phase of tiller development when the mortality ceased and the surviving tillers continued development. Age again showed more marked effects than nutrients.

The variations produced in these directions clearly indicate that growth of cane is more prominent at certain stages—the critical periods—than at others, and that nutrients altered these development phases rather to an insignificant extent. These critical periods appeared to be more or less a specific and inherent characteristic of the variety.

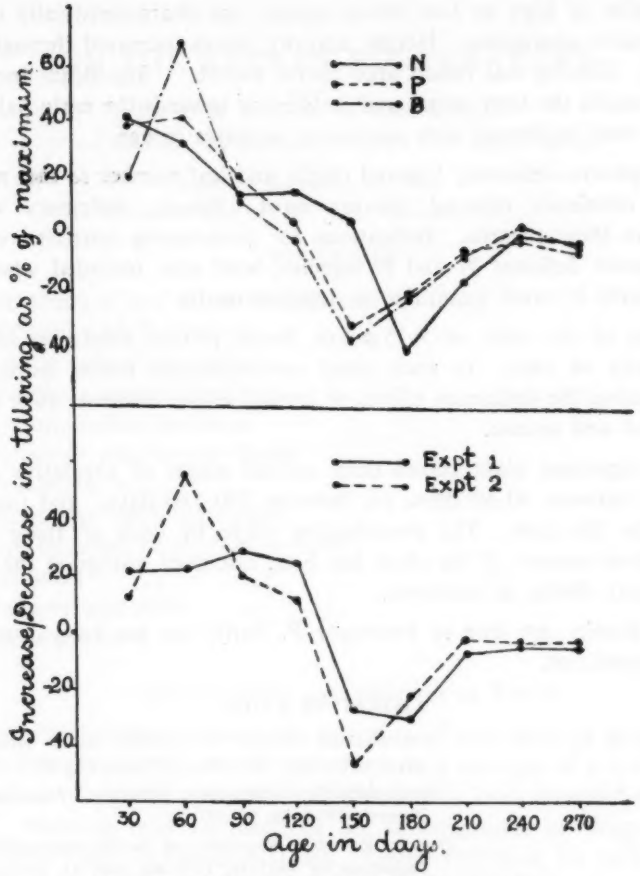

FIG. 6. Critical periods of tillering in sugarcane

SUMMARY

The paper narrates the results of investigation on the effect of certain major and minor elements upon development and vegetative vigour of sugarcane. Three series of experiments were conducted on Co 312 grown in pots under different conditions of nutrition.

Under conditions of sand nutrient cultures, boron in small doses is helpful in increasing vegetative vigour. Ten parts per million proved effective in improving both height and tillering. Nitrogen was equally effective in improving tillering while P proved to be better from height of cane point of view.

In spite of high or low boron supply, age characteristically influenced all vegetative characters. Height and dry leaves increased throughout the life-cycle; tillering was raised upto fourth month. Significant increases in height towards the later stages and in tillering towards the early half of the life-cycle were evidenced with successive increases in age.

Phosphorus deficiency lowered height and leaf number to the maximum nitrogen deficiency reduced tillering most. Potash deficiency was least harmful in these regards. Indications for overcoming restricted vegetative growth under deficient N and P supplies, were also recorded when boron was available in small quantities in deficient media.

Doses of the order of 5–15 p.p.m. boron proved helpful in improving juice quality of cane. In such small concentrations boron helped plants in overcoming the deficiency effects of certain major elements such as phosphoric acid and potash.

The sugarcane plant shows three critical stages of vegetative development: (i) between 30–60 days, (ii) between 150–180 days, and (iii) at 240 days in the life-cycle. The contribution made by each of these periods towards development of the plant has been discussed alongside the general physiological effects of nutrients.

Our thanks are due to Professor P. Parija for his keen interest and useful suggestions.

Literature Cited

1. Brenchley, W. E. .. "The essential nature of certain minor elements for plant nutrition," *Bot. Rev.*, 1936, **2**, 173–89.

2. Martin, J. P. .. "Boron deficiency symptoms in sugarcane," *Hawaiian Planters Records*, 1934, **38**, 95–107.

3. Singh, B. N. .. *Progress Reports of Researches on the Physiology of Wheat and Sugarcane for* 1937–38, 1938–40, 1940–42, Indian Council of Agricultural Research, New Delhi.

4. ———— .. "Age fertiliser effects upon certain aspects of the physiology and chemistry of sugarcane," *Proc. Ind. Acad. Sci.*, 1941, **B 13** (5).

5. ———— .. "Physiological effects of deficiency or excess of added fertilisers upon growth characters, carbohydrate metabolism yield and juice quality of sugarcane," *ibid.*, 1941, **B 15** (4),

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ON THE EYES OF POLYCHAETES

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Introduction and Historical Résum

The eyes of polychætes received the attention of zoologists as early as 1831 when Müller gave an account of the structure of the eyes of a few annelids. Descriptions of annelid eyes which followed have been meagre and unsatisfactory. Mention may be made of the investigations of Wagner (1834), Krohn (1845) and Quatrefages (1850). Greeff (1875) in his monograph of the Alciopidæ was the first to give a full account of the annelid eye. Later Graber (1880) and Carriere (1885) have given details of the structure of the eyes of various annelids but, unfortunately, they disagree on many points. Graber, as a result of his studies on the annelid eyes, has found in their structure grounds for comparison with the eyes of arthropods. The annelid eye in his conception consists of an open pigmented retinal cup closely associated with the brain and cut off from the epidermis with a mass of epidermal cells filling the cup as a refractive accessory part of the eye and not connected with the retina. The retina is composed of peculiar cell elements having at least two nuclei in each. In Carriere's estimation, the eye is a closed sac composed of the retina and the inner cornea filled by a refractive non-cellular mass that is not connected with the epidermis. The retina is a single

layer of cells in which pigmented sensory cells alternate with clear so-called secretory cells. Thus he regarded the eyes as similar to the gastropod eye. Other authors like Jourdan (1887) and Beddard (1888) have referred the eye to one or the other of these two fundamentally different interpretations. The work on the eyes of several polychætes by Andrews (1892), besides being fairly extensive, has also thrown considerable light on the probable formation of eyes in annelids. Hesse (1889) also published a similar account but differing from Andrews in certain points regarding the structure of the retinal layers in the various groups. Demoll (1909) has studied in greater detail the eye of *Alciopa cantrinii*. The latest account available on the eyes of polychætes is the one by Pflügfelder (1932) wherein he has made a careful study of the eyes in the various groups of free-living forms.

Apart from the literature cited above dealing with the simple type of eyes in polychætes, there remains a fairly large amount of facts about the so-called compound eyes as met with in Sabellids. The presence of a number of dark brown pigment spots on the branchiæ of many Sabellids has long been recorded in many systematic papers and it was Kolliker (1858) who first made a detailed study of these pigment spots in two species and suggested them to be of the nature of compound eyes. In one of them he has found that such a pigment spot was composed of fifty to sixty elements, each with a cuticle, a special refractive pear-shaped body and a crystalline cone surrounded by brown red pigment. In another, *Branchiomma dalyelli*, he has noticed eighteen to twenty pairs of hemispherical projecting pigment spots at regular intervals on the outer side of each branchia. Grube (1878) and Chattin (1878) have also made a study of these compound eyes and Chattin has attempted to compare these eyes with those of arthropods. In the systematic work of Ehlers (1887) also we meet with figures and notes bearing on the general anatomy of similar compound eyes. Seeing Nauplii entangled in the branchial filaments, he was led to doubt their function, whether they are really compound eyes or glands secreting some adhesive substance which helps in the capture of prey. But later Brunotte (1888) applying other techniques reasserted his faith in the visual function of these pigment spots in *Branchiomma*. Beddard's description of a mushroom-shaped eye at the tip of the branchia of a new species, *B. intermedium*, also supports this view. The later accounts of Andrews (1891) and that of Hesse (1899) can be looked upon as the most comprehensive treatises on the subject.

Representatives of most of the families of polychætes are available in Indian waters, yet no work has been done on the eyes of these forms. Thus it was thought that a general study of the eyes of a few typical forms together

with our existing knowledge on the subject would help to throw some light on the sequence of variations from the normal type in response to habits.

Material and Technique

The material for study was obtained mostly from Madras. The brackish-water forms were collected from near the mouths of the Adayar and the Cooum. The species investigated include typically creeping, burrowing, tubicolous, and purely pelagic forms. Specimens were cleaned and fixed in different fixatives. The heads of the worms were subsequently cut and kept in the fixatives to ensure thorough fixation of the eyes. Zenker's fluid was found to make the material too hard for satisfactory sectioning. Of the fixatives tried, Susa was found to be the best. Clearing was done in cedar-wood oil and sectioning in paraffin was resorted to throughout. In order to study the retinal structure, the retinal pigments had to be bleached. This was satisfactorily done in Grenacher's mixture or chlorine-alcohol; the former gave better results. In certain instances the pigment was bleached by treating the sections first in a 1 : 2000 solution of potassium permanganate till the sections were light brown in colour and later washing them in a solution of oxalic acid of 1 : 300 strength. The process had to be repeated till all the pigment was completely removed. Staining with Heidenhain's iron hæmatoxylin for 3–5 hours, was necessary to bring out the structures fully.

Family Eunicidæ

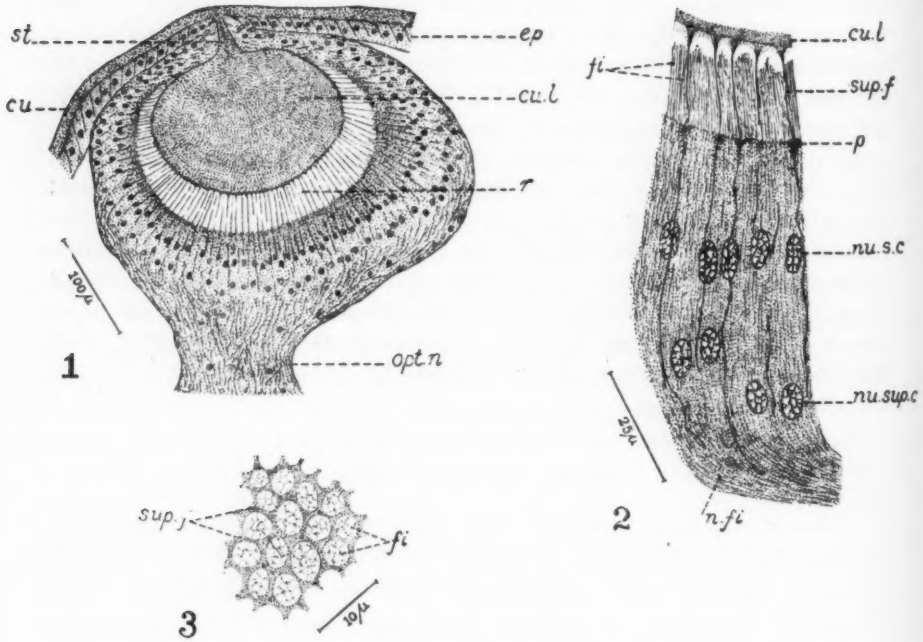
Eunice aphroditois Pallas

This is one of the creeping forms with two eyes on the prostomium, each lying at the base of an intermediate tentacle. In surface view, the eyes appear as two dark brown pigment spots and do not project much above the surface.

A median vertical section of the eye is shown in Fig. 1. In general appearance, this recalls the structure of the eye in Amphinomidæ as described by Pflügfelder (1932). The eye is almost spherical in shape, situated in the space between the hypodermis of the prostomium and the brain. Each eye is composed of a retina forming the wall of the optic cup and a central refractive body filling the cup. The eye is innervated by an optic nerve which leaves the retina at a point opposite the pupil.

Refractive body.—The refractive body fills the retinal cup. This is connected with the outer cuticle by means of a thin stalk, a fact already noted by Graber (1880), Jourdan (1887), Andrews (1892) and Pflügfelder (1932) n other Eunicids. This is a spherical mass having a structure almost like

the cuticle. In the living condition, this mass is crystalline and serves as the light dispersing part of the eye. Owing to the close similarity in structure of the refractive body with that of the cuticle, this is described as the cuticular lens. Faint concentric striations corresponding to the layers of the cuticle are noticed in this cuticular lens. The reactions of this body to various stains are also exactly similar to those of the cuticle. The lens in *Eunice* is therefore only a localised thickening of the cuticle which has gone



FIGS. 1–3. Fig. 1. A median vertical section of the eye of *Eunice aphroditois* showing the connection between the cuticle and the refractive body, 5 × 40. Fig. 2. A portion of the retina after partial depigmentation to show the detailed structure of the retinal layers, 10 × 90. Fig. 3. A tangential section passing through the region of the rods showing the fibrils and supporting fibres in the section, 10 × 90.

cu., Cuticle; *cu. l.*, Cuticular lens; *ep.*, Epidermis; *fi.*, Sensitive fibrils; *n.fi.*, Nerve fibres; *nu. s.c.*, Nucleus of the sensitive cell; *nu. sup. c.*, Nucleus of the supporting cell; *opt. n.*, Optic nerve; *p.*, Pigment; *r.*, Rods; *st.*, Stalk connecting the cuticle and the cuticular lens; *sup. f.*, Supporting fibre.

into the retinal cup, the connection between the refractive body and the cuticle persisting as a stalk. These observations are in agreement with the account given by Pflügfelder (*loc. cit.*).

Retina.—The retinal cells collectively make a pigmented epithelium forming the wall of the optic cup. The retinal cells are of two kinds, the sensitive cells and the supporting cells. The sensitive cells at their inner ends show the rods. All these rods together form a clear zone next to the cuticular lens. Fig. 2 shows a portion of the retina after partial depigmentation. The contents of the rods take only a light stain with iron hæmatoxylin. Inside each rod could be seen the sensitive fibrils having a parallel course, better revealed in fresh preparations, but these are not well seen in the region below the rods, *i.e.*, in the body of the cell. Among the sensitive cells are the supporting cells whose nuclei occupy a slightly deeper position in the retina compared to the nuclei of the sensitive cells. Supporting fibres proceed from these supporting cells which run in between the rods to the periphery of the lens where they branch into finer fibrils. These fibres stain black with Heidenhain's. The function of these fibres, as the name indicates, is to serve as supports to the other elements of the eye. Hesse (1899) considered these supporting cells to be secretory cells, but Pflügfelder has shown that they do not possess any secretory function.

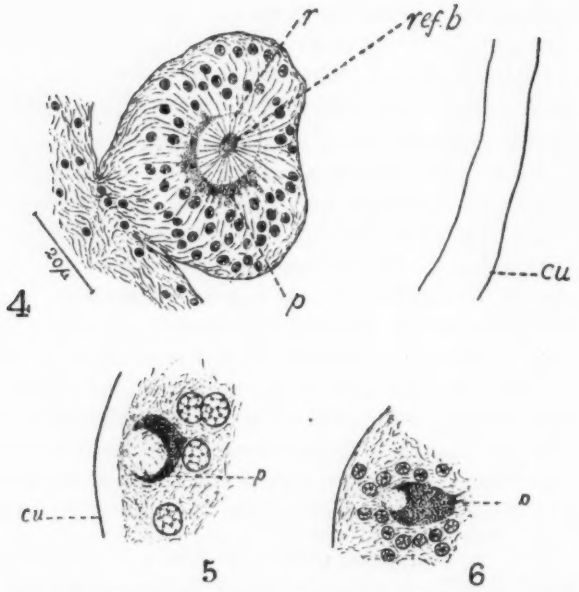
The pigment is brownish red in separate spherules and is present chiefly on the surface of the cell, densely aggregated just below the zone of rods and extends outwards becoming less dense. Pflügfelder has been able to note two types of pigments in many polychætes and he has attributed different optical behaviour to the two kinds of pigments. According to him the pigment of the supporting cells is much more dark, varying from a deep brown to a black colour whilst the pigment of the sensitive cells shows lighter colouration. But in the present form, the variation in the colour of the pigment is only apparent due to the greater accumulation of pigment granules around the supporting cells. During depigmentation the pigment granules around the sensitive cells are more easily removed because of the less marked distribution of pigment granules in this region whereas it takes a longer time to dissolve the pigment round the supporting cells.

Marphysa gravelyi Southern

Aiyar (1933) while studying the anatomy of this form has noticed the presence of a pair of eyes on the prostomium. He has also observed the fact that in very large specimens, the eyes are usually missing. However, in young forms, the eyes are visible as a pair of brown pigment spots on the prostomium at the base of the intermediate tentacles. The eyes are not very prominent as in other Eunicids.

Sections of the head of various specimens of different size were cut in different planes. In young worms the eyes are better developed than in the

adult ones where the eyes seem to be almost atrophied. Even in the young forms, the structure reveals a poor state of development. The eyes in all sections appear like a pair of closed pigment cups placed far below the epidermis. Thus they are sub-epidermal structures, both the cuticle and the epidermis lying over them being very thick (Fig. 4). The eyes are closely pressed against the brain and show a connection with it by means of a short optic nerve. The retinal cells which are comparatively few in number form the thick wall of the optic cup and have their lower ends produced into nerve fibres to form the optic nerve. The retinal cells are also pigmented, but the pigmentation is comparatively feeble corresponding to the imperfect development of the eye. Inner to the retinal cells comes the region of the rods. Supporting fibres are fairly clear among these rods while the fibrils inside



FIGS. 4–6. Fig. 4. Vertical section of the eye of *Marphysa gravelyi* to show the extremely reduced nature of the visual organs, 5 × 40. Fig. 5. T. S. of the larval eye of *M. gravelvi*, 10 × 90. Fig. 6. A similar section of the larval eye of *Diopatra variabilis*. 10 × 90. Compare with Fig. 5.

cu., Cuticle; *p.*, Pigment; *r.*, Rods; *ref. b.*, Refractive body.

the rods could not be made out. Filling the cavity of the eye is a much reduced glassy mass representing the refractive body. The structure of this refractive body could not be properly made out owing to its extremely reduced nature. It appears as a homogeneous spherical mass occupying

the space inside the retinal cup. Thus the eyes show a low degree of organisation compared to those of certain other Eunicids.

The above description of the eye of *M. gravelyi* differs from that of the eye of *M. sanguinea* Quatrefages, the only available description of the eye in this genus as given by Andrews (1892). The two eyes in this species, though deeply buried beneath the cuticle, show a greater degree of development than in the present form. Both in surface view and in vertical sections a stalk extending from the cuticle to the lens is said to be present. The lens is a clear highly refractive mass of conical form. The structure of the retina is also more definite, being composed of a number of sensitive cells and well developed rods. The pigment is dark red. Thus the eyes are better developed and show greater resemblance in their structure to typical Eunicid eyes. The causes for the difference in the eye structure in the two forms *M. gravelyi* and *M. sanguinea* are discussed in a later section.

A study of the larval eyes of this species was made from larvæ which hatched out from eggs in the laboratory. The eyes are seen as two brown pigment spots on the head region and are well formed in the young stages. When viewed under the microscope, each eye looks like a shallow pigment cup with a central clear portion. Section at 5μ of larvæ with three setigers reveals the following structure. Each eye is a small pigment cup situated on the two sides of the brain, sunk in the tissue of the cephalic ganglia (Fig. 5). The pigment spherules are brown in colour forming a shallow cup. The cavity of the pigment cup is occupied by a lightly staining mass, not much differentiated from the rest of the brain. This represents the refractive part of the eye. Even after the removal of the pigment, no special structures like retinal cells or rods could be made out in this condition. The larval eyes in these have been compared with those of *Diopatra variabilis* (Fig. 6) in which also the larvæ have eyes while the adults do not possess them. The structure of these larval eyes is also very much like that of the larva of *M. gravelyi*. In *M. gravelyi* the simple larval eyes are said to disappear at the 13 to 14 parapodial stage and their place being taken up by the eyes of the adult (Aiyar, 1931). It is therefore still a matter for investigation as to how the larval eyes degenerate to give place to the adult eyes and also how these adult eyes themselves are reduced till complete atrophy is reached.

Family Nereidæ

Lycastis indica Southern

As in all Nereids, this errant species possesses two pairs of eyes situated on the prostomium. The structure of the eye also resembles very much

that of the other Nereids where the eye is formed of a closed vesicle with a cornea of hypodermal cells and a glassy refractive body enclosed within the cup. The wall of the cup itself is made up of pigmented sensitive cells constituting the retina.

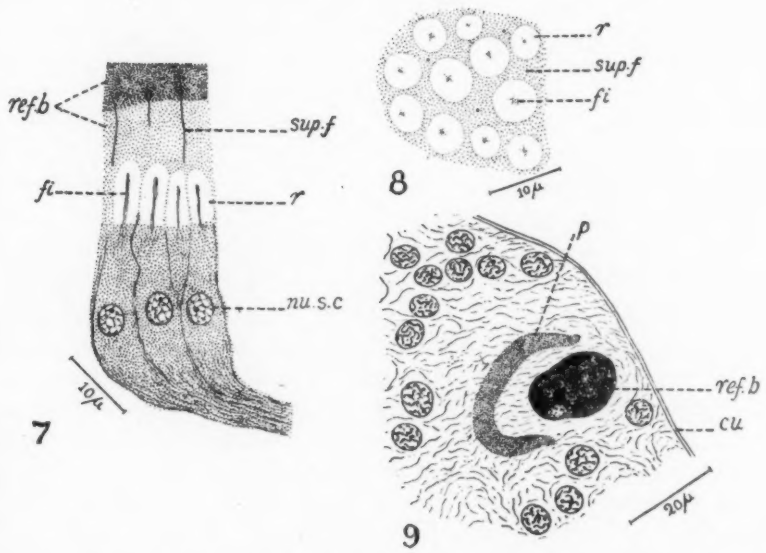
In a transverse section of the prostomium passing through the eyes, they are seen to lie close to the brain and occupy the space between it and the epidermis. The optic nerve enters the eye at a place opposite the pupil.

Retina.—The retina is a single layer made up of sensitive cells and a few supporting cells. Processes from the supporting cells pass through the vitreous body and these are the supporting fibres.

The sensitive cells have at their distal ends the rods projecting into the optic cup. Each sensitive cell has a well staining nucleus somewhere in the middle. The nucleolus stains dark with iron hæmatoxylin. These cells at their periphery show delicate nerve fibres leaving them. Such nerve fibres of all the retinal cells together get themselves entangled and form an outer covering for the eye which finally come together and converge to form the optic nerve. The retinal cells are pigmented, the pigment is especially dense along a line sharply separating the cells from the clear rods and thence extends to the periphery somewhat regularly. Regarding the formation of pigment, it is believed to be produced by the retinal cells. The structure of the rods is not very different from that in other Nereids. They project from the distal ends of the sensitive cells into the glassy crystalline mass filling the optic cup. The walls of these rods are thin but firm and the rods have a lightly staining cytoplasm. A large number of fibrils, which apparently lie in a bundle, traverse the length of the rods (Fig. 7). The tips of these fibrils take up a deeper stain. The thin walls of the rods protect these delicate sensitive fibrils. A tangential section of the eye at the level of the rods (Fig. 8) shows the structure more clearly.

The refractive body.—The optic cup is completely filled with the semi-solid crystalline refractive body. In some Nereids and also in a species of *Lycastis* worked out by Pflügfelder (1932) this refractive body is found to be made up of two distinct regions, a solid round lens-body and a jelly-like vitreous body lying in the space between the lens and the retina. Depending upon the origin of these two, Pflügfelder prefers to call the first as the cuticular lens-body (cuticuläre Linsenkörper) and the other as the vitreous body (glaskörper). The present observations in *L. indica* differ from this. The refractive body filling the optic cup throughout appears to be a jelly-like mass in which are seen the projecting ends of the supporting fibres as well as

the longitudinal striations which start from the periphery and converge to a region near the cuticle. Though this mass cannot be differentiated into a cuticular lens and a vitreous body, two separate zones can easily be made out, a lightly staining narrow zone near the region of the rods and a more darkly staining central part which shows the characteristic longitudinal striations. This central darkly staining region, however, seems to be a viscous mass even in well fixed material and in a few cases, irregular empty spaces also have been formed inside this mass, perhaps due to the contraction of the viscous body. The refractive mass does not show a solid structure and I am led to conclude that the refractive body in this species of *Lycastis* is not differentiated into a cuticular lens and a vitreous body.



FIGS. 7–9. Fig. 7. A part of the retina of *Lycastis indica* after depigmentation, 10×90. Fig. 8. A tangential section of the same passing through the rods, 10 × 90. Fig. 9. Section of the eye of *Tomopteris sp.* to show the simple nature of the retinal cup, 10 × 90.

cu., Cuticle; *fi.*, Sensitive fibrils; *nu. s.c.*, Nucleus of the sensitive cell; *p.*, Pigment; *r.*, Rods; *ref. b.*, Refractive body; *sup. f.*, Supporting fibre.

Cornea.—The cornea consists merely of the usual unmodified epidermal cells and the layer of cuticle over it. The cuticle shows no variation from that covering the surface. No connection between the cuticle and the refractive body could be proved to exist, thus differing from the Eunicid eye.

Perinereis aibuhitensis Grube

There are two pairs of eyes which for all external appearance resemble the eyes of *Lycastis indica*. Both pairs are of the same size and structure. The eye in this is also formed of an epidermal cup which has become separated from the epidermis. The cuticle and a layer of hypodermal cells pass over the eye, both together forming the cornea. The cuticle covering the eye is not differentiated from that on the rest of the body surface, the hypodermal cells also do not show any specialisation in structure. They are however slightly more transparent in the region above the eyes and take only a lighter stain.

The refractive body has the same structure as that in *Lycastis*, with radial striations proceeding from the retina and passing on to the region near the cornea. It almost fills the optic cavity and a differentiation of this refractive body into the cuticular lens and the vitreous body is not apparent. It seems to be composed of fibres running parallel to one another which are held together by a binding substance. The whole refractive body has thus an irregular appearance owing to its semi-solid nature. It does not show any connection with the cuticle and its structure is also different from that of the cuticle. It is more or less like the vitreous body found in the eyes of other polychætes. The whole of this refractive mass takes stain almost to the same extent except in a very narrow zone next to the region of the rods which stains light blue.

The retina also has the same structure as in *Lycastis*, being composed of a layer of sensitive and supporting cells. The rods are borne at the inner ends of the sensitive cells. A bundle of fine fibrils lies in the middle of each rod. The supporting fibres of the supporting cells are clearly seen passing into the refractive body. The pigment is of deep blue colour. Outside the retina and covering the optic cup are a few large prominent nerve cells. These have a clear appearance, each with a well staining nucleus. The nucleolus also stains deeply. The special function and significance of these nerve cells are not known at present.

Family TOMOPTERIDÆ

Tomopteris sp.

This pelagic polychæte has two eyes situated on the head, seen as two small brown structures in the living condition. A careful study of the eyes in the living animal reveals the presence of a clear transparent crystalline body in each eye which projects out from the pigment cup. This is clearly visible owing to the transparent nature of the animal.

The two eyes are buried in the brain. Their structure appears very simple as seen in a transverse section (Fig. 9). In each we could recognise the glassy refractive body, nearly spherical and situated in the pigment cup, staining deeply with iron hæmatoxylin. Andrews (1892) noticed that the refractive body in *Tomopteris* had a granular structure. But in the present material the refractive body shows a definite shape and crystalline glassy nature without showing any granular structure. Perhaps it may be due to the improper preservation of the material which Andrews had examined. Between the refractive body and the pigment cup is a clear zone corresponding to the region of the rods although no rods have been observed. The retina also does not have any clear cellular structure. Well defined retinal cells appear to be absent. However, a few large round nuclei situated outside the pigment cup may represent the nuclei of the retinal cells. The pigment granules are brown in colour and form a cup-like structure surrounding the refractive mass.

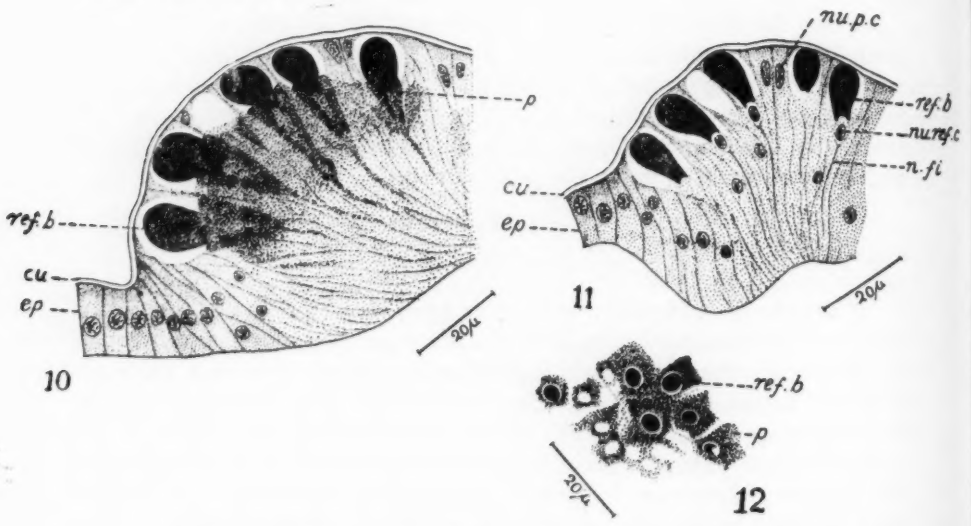
From the above description it may be seen that the structure of the eyes resembles that of the Spionidæ given by Hesse (1899). In both the retina is very simple, being formed of a simple pigment cup embedded in the brain without any well built retinal cup. But in *Tomopteris* the refractive body is well developed, whereas in Spionidæ, this is represented only by a mass of undifferentiated tissue inside the pigment cup.

Family Sabellidæ

Dasychone cingulata Grube

The eyes of *D. cingulata*, as in most Sabellids with eyes, are situated on the branchial filaments in the form of small paired red-brown pigment spots on the lateral sides of the main stem of the branchiæ. There are about ten to fifteen paired eye spots occurring between each pair of dorsal stylodes. Each eye spot is a small hemispherical projection on the surface of the branchial stem. The surface of these elevations appears to be homogeneous but when the microscope is focussed a little below the surface, a number of clear rounded bodies can be seen just above the pigment region. These crystalline bodies are the components of the compound eye; they are the light refractive bodies. The general lining of the cuticle extends over these eyes also; it does not seem to show any differentiation in the region of the eye. Each eye is composed of several individual cells and these cells are essentially of two types, the refractive cells and the pigment cells. An eye in a transverse section is shown in Fig. 10. There are about twenty to thirty refractive cells, only five such cells are seen in the figure. Their structure could be made out in maceration preparations. Each cell has a highly

refractive body at its broader end and lies just below the outer layer of cuticle. These do not show any connection with the cuticle unlike the condition in *D. conspera* described by Andrews (1891). These crystalline bodies take up a dark colour with most of the stains like hæmatoxylin and borax carmine. These have a clear appearance and do not show any structural peculiarity and these serve the purpose of the lens. The narrow inner ends of these bodies have slight depressions and the nucleus of the refractive cell is placed near this end (Fig. 11). The nucleus has an almost oval shape and does not stain very dark. The inner ends of the refractive cells are continued as slender fibrils. Proper staining of these fibrils can be done by keeping sections in Heidenhain's hæmatoxylin for about six hours and then destaining them. The nerve fibrils are seen to merge into the sub-epithelial nervous system without forming any definite optic nerve. In *Potamilla*, Andrews (*loc. cit.*) has been able to observe the formation of a nerve starting from the eye.



FIGS. 10–12. Fig. 10. Vertical section of the compound eye of *Dasychone cingulata*, 10 × 90. Fig. 11. The same, after depigmentation. In both the individual units and a few refractive bodies are seen, 10 × 90. Fig. 12. A tangential section passing through the base of the refractive bodies, 10 × 90.

cu., Cuticle; *ep.*, Epidermis; *n. fi.*, Nerve fibres; *nu. p. c.*, Nucleus of the pigment secreting cell; *nu. ref. ce.*, Nucleus of the refractive cell; *p.*, Pigment; *ref. b.*, Refractive body.

During maceration, the eyes break up into a number of these refractive bodies and most of them are covered by small spherules of brown pigment.

These pigment granules surrounding them could be easily removed by slight pressure under the cover glass. The position of pigment in the eye is made out in sections that are not depigmented. In materials fixed in light, the pigment surrounds the refractive cell, but does not extend to the very outer surface. The pigment starts at about the middle of the refractive body and extends for a great part into the eye so that these bodies appear as though they are partly immersed in a mass of pigment (Fig. 10). In a tangential section of the eye through the pigment zone (Fig. 12) the pigment is seen surrounding each refractive body. The pigment cells are slender, elongated and placed among the refractive cells, but there seems to be no special arrangement of these cells. They occur irregularly among the other cells and not definitely between them being without any precise number or position. These cells have a more elongated nucleus and are thus readily distinguished from the refractive cells, both by the absence of the refractive bodies and by their slender appearance. Sometimes even in sections the cell boundaries of these cells cannot be clearly made out.

Transverse sections of the branchiæ through the eyes show that they are symmetrically placed on the outer sides of the branchial stem. The eyes seem to be a modified thickening of the epithelial cells, the cuboidal cells of the epithelium gradually become elongated near the eye and in the eyes the cells are elongated to their maximum. As in the epidermis, in the eye also there is only one layer of cells, but more closely packed. The cells are broader towards the periphery and appear to radiate from the centre of the eye. Some of the cells bear crystalline bodies and a centrally situated nucleus while others are slender and more elongated, distributed among the first type of cells. The former is responsible for light refraction while the latter type functions as the pigment secreting cells.

Eye Structure in Relation to Habits

From the above account and also based on previously known facts regarding the structure of the eye in polychætes, certain remarks of a general nature can be made particularly about the sequence of variation in the different types in response to their various habits and it might be seen that the degree of development of the visual organs is associated with the mode of life of the animal.

Among the Errantia which includes the Amphinomorpha and the Nereimorpha (Rapacia)[1] we find that eyes are present in most of the families.

[1] The classification by Hempelmann given in *Handbuch der Zoologie*, Kükentha and Krumbach (1934) has been adopted.

But there are also a number of forms where eyes are either partially reduced or even completely absent in the adult condition. Various stages in the transition from the well developed eyes to the completely atrophied condition may be noted among the errant forms. Usually the deterioration of the eye may be correlated with the difference in habits of the animals. In either instance where the adults have poorly developed visual organs or have no eyes at all, the free swimming larvæ often possess eyes and the deterioration of the eyes sets in as these larvæ take up a burrowing or tubicolous mode of life.

The family Eunicidæ includes animals with varying habits. A number of them like *Eunice aphroditois* are creeping forms, a few like *Diopatra* are tubicolous and others like *Marphysa* are burrowing in habits. In their free-living larval period all these possess eyes. But later as further development proceeds, these larval eyes are replaced by the eyes of the adult. In several species of *Eunice* the eyes are well developed with a retinal layer composed of sensitive cells with rods and supporting fibres among them. The retinal cup encloses within it a refractive body derived from the cuticle and which retains a connection with it. This typical structure is seen in most species of *Eunice* which lead a free or creeping mode of life. Among the tubicolous and burrowing forms, we are able to notice a great difference in the structure of the eyes. They are either poorly developed or even absent in the adults. In these also as in others, the larval eyes are equally well developed having a simple pigment cup enclosing a clear refractive part. But in the adults well-built eyes are lacking owing to the mode of life assumed by them. *Marphysa sanguinea*, has eyes which in the adult show a structure almost resembling the typical Eunicid eye as described before. But the retina is less developed and encloses the cuticular refractive body. In *M. gravelyi* which is also a burrowing form, the eyes are much more reduced. The whole eye is sunk far beneath the epidermis and shows a comparatively low degree of organisation.

A few of these worms were brought to the laboratory and experiments performed on them for phototropism gave negative results. A long glass tube open at both ends, of 20 inches in length and 1½ inches diameter, was divided into two compartments by covering one half with black paper so as to form a dark chamber and the uncovered part was illuminated by direct light from a 60 watt lamp at a distance of about 2 feet. The tube was kept perfectly horizontal and the worms were introduced into the illuminated part of the tube with enough water to keep them moist. Particular care was taken to see that the temperature of the water in the tube was kept almost constant lest it should have any effect on the movement of the animals inside

the tube. The worms thus kept in the illuminated chamber of the tube gradually moved on to the dark portion of the tube where they remained indefinitely. Those which were first introduced into the dark chamber did not move away at all and remained in the darkness. The results were almost consistent with all the ten worms tried except in one instance where the movement was erratic. Similar experiments were performed with younger specimens in which the eye spots were carefully burnt with a hot needle and the removal of the eyes was thus ensured. Although it took some time for them to recover from the shock of the operation, they behaved quite normally after a while like those of the first series preferring darkness to light. These experiments while showing their negative phototropism, also throw some doubt on the efficiency of these imperfectly developed eyes as organs of light perception.

In the literature the question has often been discussed whether the absence of eyes in certain Eunicids is a primary or secondary phenomenon. Andrews (1892) remarks that cases in which eyes are found buried in the brain and separate from the surface are to be considered secondary, either the results of degenerative reduction from the typical type or retention of larval characters with the addition of separation from the cuticle. Later, Pflügfelder (1932) finds that in *Diopatra amboinensis* eyes are always lacking in the grown-up animal, but are present in the early young stage. In structure, they resemble the eyes of all other Eunicids. So long as these animals are free living (*i.e.*, remaining in the tube of the mother animals), the eyes are present but disappear after the animals have built their own tubes. In the place of the eyes are found brown concretions in whose neighbourhood no sensory cells are to be seen. Pflügfelder after studying the conditions in both the larvæ and the adults, also states that the absence of eyes in many Eunicids is only a secondary disappearance which is probably brought about by the sedentary mode of life. The same might be true of *Diopatra variabilis* where the larvæ possess eyes while the adults do not have them. Sections of the adult animals do not show the presence of any vestigial eyes even in a hidden condition.

Taking another family Nereidæ, we are able to note the same phenomenon of variation in the eye structure according to the habits of the animal. But the difference is not so pronounced, probably owing to the fact that all Nereids have more or less the same active habits. The family consists of free living errant forms, mostly creeping among stones and debris. All of them possess prominent eyes usually four in number arranged on the prostomium. Each eye is composed of pigmented retinal cup, lined by clear rods and filled by a refractive substance. All the larval Nereids also possess

eyes which show the simple structure, having a small pigment cup with a central refractive body. The larval eyes are often more than one pair, sometimes even three pairs while the posterior four are usually permanent and the anterior two are only the provisional eyes. Even though animals belonging to this family have almost the same habits, it has often been noted that many Nereids are slowly getting adapted to a terrestrial mode of life. Several species of *Lycastis* are examples of this for which reason they have been called " Earth Nereids " by Harms (1929) and Pflügfelder (1932). Ranganathan (1942)[2] has found that *L. indica* which is able to maintain itself successfully in terrestrial surroundings the body is specially organised for this purpose. These annelids are said to possess special cutaneous and respiratory adaptations to stand a certain amount of drought. The eyes in *Lycastis* though four in number like all Nereids, have a thick layer of cuticle and epidermis over them. In the retinal layer also there is a slight difference in that the rods and the supporting fibres are fewer in number than those in Eunicidæ.

In contrast to the creeping and burrowing forms, if we consider the typical pelagic polychætes like Alciopidæ and Tomopteridæ we notice certain interesting features. In both, the eyes are present in the larvæ as well as in the adults. Though both lead a planktonic life, the degree of development of the eyes in these two groups shows a marked contrast. In *Tomopteris* the eyes are small, each composed of a pigment cup buried in the brain. The retina is thus very simple without being differentiated into a distinct layer of cells. The refractive body is situated in the pigment cup and separated from it by a clear zone representing the rod-like ends of a few large cells surrounding the eye. About the structure of these eyes, Andrews (1892) has said, " The simple character of the eyes in this family of pelagic annelids, as compared with the perfect forms found in the free swimming Alciopidæ, Nereidæ and Syllidæ may possibly be connected with the great transparency of the body in *Tomopteris*, that rendering a projecting convex eye less necessary ". In Alciopidæ we find perhaps the highest organisation of the visual organs among polychætes. The large prominent eyes in this family have been the subject of investigation by workers like Demoll (1909). In the adult Alciopids, the eye consists of a well-developed retinal layer of sensitive cells bearing rods at their inner ends. There is a clear lens between which and the retinal layer is a vitreous body. These eyes are even said to be capable of adapting themselves to variations in light. From Béraneck's (1893) embryological investigations of *Alciopa*, we are informed that the lens

[2] Unpublished.

arises from a crystalline cell (" Cellule crystallinienne ") in the centre of the eye which later grows during development. The structure and formation of the eyes of Alciopidæ are unique showing a most highly organised type of visual organ adapted for pelagic mode of life.

Considering the case of sedentary polychætes, particularly the Serpulimorpha, we find a gradual evolution of the visual organs in certain groups which are slowly becoming positively phototropic. As in the case of errant forms, usually larval eyes are present in these sedentary polychætes during their free swimming period. Eyes have been figured in the larvæ of Sabellaridæ and Sabellidæ (*Branchiomma*) by Wilson (1929 and 1936). As the larvæ settle down and metamorphose into the adult to take up a tubicolous habit, the cephalic eyes disappear. Their place is taken up by the branchial eyes in the case of the Sabellidæ while in the Serpulidæ and Sabellaridæ usually eyes are not present in the adults. Both these species are, however, highly sensitive to light. In *Protula protula*, a group of pigmented cells occur on the branchiæ which Hesse (1899) believed to be visual in function. Visual organs are much better developed in Sabellids even though there are a few exceptions like *Sabellastarte* and *Dasychone serratibranchis* which do not possess these branchial eyes. The simplest type of eye is seen in *Hypsicomus* (Andrews, 1891) consisting of a single cell containing a refractive cuticular ingrowth. A few pigmented cells also surround this central refractive cell. From its structure, it is considered as a modification of the epidermal cell. In others like *Potamilla* and *D. cingulata*, each branchial eye is composed of a cluster of similar sensitive cells, each with a refractive body in it. The pigment cells are disposed among the sensitive cells. Thus the structure of these eyes is more elaborate than that of the simpler type in *Hypsicomus*. A comparison of the larval and adult eyes can be made only after we have a fuller knowledge of the eyes of the Sabellid larvæ.

Summary

1. A detailed study of the eyes of a few common polychætes of Madras with different habits has been carried out giving a brief comparison of their structure with that of allied forms from European waters.

2. The eye in Eunicidæ is usually composed of a retinal cup and a central refractive body which consists of a localised thickening of the cuticle; the connection between the cuticle and the refractive body being retained in the adult worms.

3. In Nereidæ which includes mostly free living errant forms, the refractive part of the eye consists of a more or less semi-solid glassy body which does not show any connection with the cuticle.

4. In the Tomopteridæ which are pelagic the retina is a simple pigment cup without differentiation into retinal cells or rods. The nature of the refractive body is also very simple.

5. In Sabellids which are tubicolous the visual organs are of the nature of compound eyes occurring on the main stem of the branchiæ. Each of these is composed of a number of individual units with a separate refractive body.

6. Although polychætes of the same family have almost the same ground plan in the construction of their eyes, differences in the degree of development of the eyes have been observed within the same family. The causes of these differences in the visual organs have been attributed to the differences in the mode of life of the animals and it has been found that the free living or errant forms have much better developed eyes compared with the burrowing ones where the eyes are usually absent. In all of them the early free swimming larvæ possess eyes but in the case of burrowing forms the eyes disappear as they grow, often leaving no trace of their existence in the adult condition.

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BIBLIOGRAPHY

Aiyar, R. G. .. "Development and breeding habits of *Marphysa*," *Journ. Linn. Soc.*, 1931, **37**.

———— .. "On the Anatomy of *Marphysa gravelyi* Southern," *Rec. Ind. Mus.*, 1933, **35** (3).

Andrews, E. A. .. "On the Eyes of Polychæta (A Preliminary Communication)," *Zool. Anz.*, 1891, **Bd. 14**, No. 371.

———— .. "Compound eyes of Annelids," *Journ. Morph.*, 1891, **5**.

———— .. "Eyes of Polychætous Annelids," *Ibid.*, 1892, **7**.

Beddard, F. E. .. "Reports on Annelids from the Mergui Archipelago," *Journ. Linn. Soc.*, 1888, **21**.

Béraneck, Ed. .. "Embryogénie et Histologie de l'Oeil des Alciopides," *Revue Suisse de Zoologique*, 1893, T. 1.

Brunotte, C. .. "Recherches sur la structure de 'oeil chez un *Branchiomma*," *Comptes Rendus*, 1888, **1**, 106.

*Carriere, J. .. "Die Sehorgane der Thiere," *Munchem u. Leipzig*, 1885.

Chatin, J. .. "Recherches pour servirá l'histoire du batonet optique," *Ann. Sci. Nat. Zool.*, 1878, Ser. 6, T. 7.

Demoll, R. .. "Die Augen von *Alciopa cantrainii*," *Zool. Jahrb. Anat.*, 1909, **Bd. 27.**

Ehlers, E. .. "Reports on Annelids," *Mem. Mus. Zool. Harv.*, 1887, **25.**

Fauvel, P. .. "Polychetes Errantes," *Fauna de France, Paris*, 1923.

———— .. "Polychetes Sedentaires," *Ibid.*, 1927.

———— .. "Annelida Polychaeta of the Indian Museum, Calcutta," *Mem. Ind. Mus.*, 1932, **12** (*a*).

Graber, V. .. "Morphologische Untersuchungen uber die Augen der freilebenden marinen Borstenwurmer," *Arch. Mikr. Anat.*, 1880, **Bd., 17.**

*Greeff, R. .. "Über die Augen insbesondere die Retina der Alciopiden," *Sitzgsber. Ges. Beforder. ges. Natw. Marburg.*, 1875, 3–24.

*Grube, E. .. "Annulata Semperiana," *Mem. Acad. Imper. Sci. St. Petersburg*, 1878, **7**, 25 (*a*).

Harms, J. W. .. "Die Realisation von Genen und die consecutive adaptation," *Zeit. f. wiss. Zool.*, 1929, **Bd. 133.**

Hesse, R. .. "Untersuchungen über die Organe der Lichtempfindung bei niederen Tieren, 5, Die Augen der Polychaten Anneliden," *Ibid.*, 1899, **Bd. 65.**

———— .. "Untersuchungen über die Organe der Lichtempfindung bei niederen Tieren. Weitere Tatsachen Allgemeines," *Ibid.*, 1902, **Bd. 72.**

Jourdan, Et. .. "E'tudes histologiques sur deux especes du genre *Eunice*," *Ann. Sci. Nat.*, 1887, Ser. 7, **2** (*b*).

Kolliker, H. .. "Über kopfkiemer mit Augen an den Kiemen," *Zeit. f. wiss. Zool.*, 1858, **Bd. 9** (*b*).

Krohn, A. .. "Zoologische und anatomische Bemerkungen uber die Alciopiden," *Arch. f. Natg.*, 1845, 11.

Kükenthal, W., and Krumbach, T. *Handbuch der Zoologie*, 1934, **Bd. 2**, Pt. 2.

Müller, J. .. "Memoire sur la structure des yeux chez les Mollusques, Gastropods et quelques Annelides," *Ann. des. Sere. Nat.*, 1831, T. 22.

Pflügfelder, O. .. "Über den feineren Bau der Augen freilebenden Polychaten," *Zeit. f. wiss. Zool.*, 1932, **Bd. 142.**

Quatrefages, A. .. "E'tudes sur les types inferieures de l'embranchement des Anneles—Memoire sur la famille des Polyophthalmiens," *Ann. Sci. Nat.*, 1850, 3*rd Ser.* **T. 13.**

Ranganathan, V. .. "On an extreme case of adaptation in a Polychaete *Lycastis indica* Southern etc." (*Unpublished*), 1942.

Wagner, R. .. *Lehrbuch der vergleichenden Anatomie*, 1834, **Bd. 1.**

Wilson, D. P. .. "The larvae of British Sabellarians," *Journ. Mar. Biol. Assn.*, 1929, **16**, No. 1.

———— .. "The development of *Branchiomma vesiculosum*," *Quart. Journ. Micro. Sci.*, 1936, **78**, Pt. 4.

* These were not referred to in original.

TOXICITY OF PTERYGOSPERMIN AND ALLICIN

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Introduction

The majority of the preparations introduced in chemotherapy appear to be effective mostly against Gram-positive infections. During the course of our systematic investigations on plants as sources of antibiotics it was found that many of them contained anti-bacterial principles active against Gram-negative pathogens.[1] Two of these pterygospermin[2] from drumstick roots and allicin[3] from garlic have been isolated. They have also been shown to inhibit the growth of acid-fast micro-organisms. Detailed investigations to find out the efficiency of these compounds as chemotherapeutic agents were hence undertaken, and in the present communication we describe the results of our studies on the toxicity of the drugs to laboratory animals.

Experimental

Materials

Pterygospermin prepared in this laboratory by the method described by one of us[2] was used for these experiments. The method consists in adsorbing the antibiotic from alcoholic extract of the root on norit charcoal, eluting from the adsorbent with light petroleum and removing the petroleum in vacuum. Pterygospermin so prepared and used had an activity of 250 to 300 pterygospermin units per mg.

The preparation of allicin has also been described by one of us.[3] In brief it consists in the following. Alcoholic extracts of garlic are concentrated in vacuum. This alcohol concentrate is shaken well with chloroform, when allicin passes into the chloroform layer. Allicin is detained on removal of chloroform of vacuum and when so prepared it inhibited the growth bacteria at a dilution of 1 in 50,000. The antibacterial activity exhibited by both allicin and pterygospermin against a few Gram-positive, Gram-negative and acid-fast micro-organisms is given in Table I (since the detailed antibacterial spectrum has been presented elsewhere it is not given here).

TABLE I

Antibacterial properties of pterygospermin and allicin

Name of organism	Minimum inhibiting concentrations of	
	Pterygospermin in units per ml.*	Allicin in mg. per ml.
1 *B. subtilis* ..	1	20
2 *S. aureus* ..	1	20
3 *E. coi.* ..	4·5	20
4 *B. typhosus* ..	3·0	20
5 *B. dysenterieae* ..	1·0	20
6 *Mycobacterium tuberculosis* ..	1·0	20

* A pterygospermin unit has been defined by us as that amount of the antibiotic which when present in 1 ml. of sterile nutrient broth at pH 7 just inhibits the growth of a given strain of *S. aureus* for a period of 24 hours incubation at 37°C. the inoculum being 0·01 ml. of the 24 hours broth culture of the organism. This has been found to be equivalent to 3·5 μgs of pterygospermin.

Animals

Young healthy mice weighing about 20 to 25 grams and rats of approximately 90 gm. weight were used for these studies. A total of about 250 animals have been used. The animals were maintained on a balanced diet with water available at all times.

Methods and Results

Effect of tissue fluids on antibiotics.—Tissue fluids either by their enzyme activity, or by their ability to adsorb antibacterials or by their reaction have been often found to inhibit the antibacterial activity of several drugs. Experiments were hence carried out to find the effect of blood, gastric and pancreatic juice on the activity of the antibiotic substances.

The experimental procedure adopted consisted in mixing known amounts of pterygospermin and allicin, with sterile water, sterile citrate solution, citrated blood, artificial gastric juice and artificial pancreatic juice, incubating the mixtures at 37° C. and finding their antibacterial activity at definite intervals of time by the serial dilution or cylinder-plate method.

The artificial gastric juice and pancreatic juice used were prepared according to the method described by Maney and Kuever.[4] Fresh citrated human blood was used. In Table II below the results of these studies are given.

TABLE II

Effect of tissue fluids on the activity of pterygospermin and allicin

Name of medium	Activity after ( ) hours													
	Pterygospermin							Allicin						
	0	2	4	6	8	10	24	0	2	4	6	8	10	24
1. Sterile Water	18	18	18	17	17	17	17	31·0	31·0	31·0	30·0	29·5	29·5	29·0
2. Sterile Citrate	18	18	18	17	18	17	18	31·0	31·0	31·0	29·5	30·0	29·5	29·0
3. Citrated blood	18	18	18	17	17	17	17	31·0	31·0	31·0	31·0	31·0	30·5	29·5
4. Gastric juice	18	18	18	17	17	18	17	31·0	31·0	30·0	30·0	29·5	29·5	29·0
5. Pancreatic juice	18	17	16	15	11	8	4	31·0	27·0	26·0	24·0	20·0	16·0	nil

In Table II the results for pterygospermin are given in 'pterygospermin units', and for allicin the figures are the halo-diameters in mm. obtained by finding out the activity by the cup-plate method. It is easily seen from the table that the activity of both the antibiotics is lost only in presence of pancreatic juice and not in presence of other tissue fluids studied. Control experiments carried out however show that the reaction of the medium is mainly responsible for this.

Toxicity Studies

Since both the antibiotics were found to be destroyed in presence of pancreatic juice, the effect of administering the drugs orally was not studied. Subcutaneous acute and cumulative toxicity of both allicin and pterygospermin were studied. Acute intraperitoneal toxicity was studied for allicin alone. Since both the drugs were not soluble in pure water, freshly prepared emulsions of the alcoholic solutions in water were used for the injections.

Acute toxicity.—The following experimental procedure was employed. Rats were used for allicin and mice for pterygospermin. The animals were divided into a number of groups, each group consisting of six animals. All the animals in one group received the same amount of the drug, the amount of drug administered for different groups being different. The total volume of the emulsions injected were 0·25 ml. for rats and 0·20 ml. for mice. Massive doses of the drug were given in one injection for acute toxicity. The animals were then kept under observation for a period of five to seven days.

Experiments were repeated with more animals for groups in which mortality rate was high. Animals dying during the period of observation were dissected and a gross examination of the more important body organs carried out. In Tables III and IV the results of these investigations are brought out.

TABLE III

Acute subcutaneous toxicity of pterygospermin

Dose in mg./kg.	Number of mice tested for each dose	Total number of animals dead time in days					
		1	2	3	4	5	6
1. 5	6	0	0	0	0	0	0
2. 10	6	0	0	0	0	0	0
3. 50	6	0	0	0	0	0	0
4. 100	6	0	1	1	1	1	1
5. 200	10	0	1	2	2	2	2
6. 300	30	9	13	15	15	15	15
7. 400	15	5	8	11	12	12	12

TABLE IV

Acute subcutaneous toxicity of allicin

Dose in mg. per kg.	No. of rats tested	Total number of rats dead time in days					
		I	2	3	4	5	6
1. 10	6	0	0	0	0	0	0
2. 20	6	0	0	0	0	0	0
3. 30	6	1	1	2	2	2	2
4. 50	6	1	2	3	3	3	3
5. 75	8	3	4	5	6	6	6

Acute toxicity for allicin is found to be much higher than that for pterygospermin. Fifty per cent. mortality occurred in the former at 50 mg. per kg. whereas in the latter 300 mg./kg. were required to create the same effect. Dissection of the dead animals revealed that other than slight necrosis at the site of injection there was nothing abnormal.

Acute intraperitoneal toxicity was determined only for allicin. The general experimental procedure was the same as the one described above for subcutaneous toxicity. The administering of the drug in this case was done intraperitoneally. The results are given in Table V.

TABLE V

Intraperitoneal acute toxicity of allicin

	No. of mice	Dose in mg./kg.	Total number dead after days						
			1	2	3	4	5	6	7
1.	6	15	0	0	0	0	0	0	0
2.	6	20	1	2	2	3	3	3	3
3.	6	30	1	3	3	5	5	5	5
4.	6	Nil	0	0	0	0	0	0	0

Examination of the dead in the above experiments however indicated nothing abnormal. The results presented in Table V show that the intraperitoneal L.D.$_{50}$ for allicin is about 20 mgm. per kg.

Cumulative toxicity.—The effect of repeated daily administration of the two antibiotics was also determined on a group of mice. Small amounts of pterygospermin and allicin were injected subcutaneously daily for a period of ten days. The amount of emulsion injected and other experimental details were the same as those previously described. The results are brought out in Tables VI and VII.

TABLE VI

Cumulative toxicity of pterygospermin

Group No.	Dose in mg./kg. per day	No. of mice used	Total number of mice dead after ( ) days									
			1	2	3	4	5	6	7	8	9	10
1	35	6	0	0	0	0	0	0	0	0	0	0
2	45	6	0	0	0	0	0	0	0	0	0	0
3	55	6	0	2	3	3	4	5	6	6	6	6
4	85	6	0	2	3	4	5	6	6	6	6	6
5	115	6	2	3	4	5	6	6	6	6	6	6
6	Nil	6	0	0	0	0	0	0	0	0	0	0

TABLE VII

Cumulative toxicity of allicin

Group No.	Dose in mg./kg. per day	No. of mice used	Total number dead after ( ) days									
			1	2	3	4	5	6	7	8	9	10
1	0·75	6	0	0	0	0	0	0	0	0	0	0
2	1·50	6	0	0	0	0	0	0	0	0	0	0
3	2·50	6	0	0	0	0	0	0	0	0	0	0
4	3·00	6	0	0	1	1	1	1	1	1	1	1
5	5·00	6	1	2	3	3	3	3	3	3	3	3
6	6·25	6	2	3	4	5	5	5	5	5	5	5

The results given in the above tables clearly indicate that the cumulative toxic dose (required to kill fifty per cent. of the animals) of pterygospermin is very much more than that of allicin.

DISCUSSION

Foregoing results reveal interesting and useful facts regarding the possibility of pterygospermin and allicin achieving chemotherapeutic importance. Since the experiments were carried out to assess the advisability of using the drugs in infections like those caused by Mycobacterium tuberculosis, where prolonged treatment is necessary, the cumulative toxicity of the antibiotics are much more important than their acute toxicities. Mice did not survive daily injections of even 5 mg./kg. of allicin whereas they tolerated even up to 45 mg./kg of pterygospermin. In previous communications from this laboratory[3] it has been shown that allicin is unstable unlike pterygospermin. Further, the data presented in Table I show that the antibacterial properties of pterygospermin particularly against the acid-fast organisms are much higher than those exhibited by allicin. All these facts suggest the possibility of pterygospermin achieving much more importance in chemotherapy than allicin. Investigations now in progress regarding the efficacy of pterygospermin against different bacterial infections, it is hoped, will throw more light on the exact rank which pterygospermin is likely to occupy among antibacterials.

A marked difference exist in the acute toxicity of the two compounds. Moreover, the massive dose of allicin which animals are able to withstand

is high enough. It is therefore hoped that allicin may be of use in curing infections where a single injection may be adequate.

Conclusions

1. The cumulative subcutaneous $L.D._{50}$ of allicin is 5 mg./kg. and its acute subcutaneous toxicity 50 mg./kg.

2. The intraperitoneal toxic dose of allicin at which 50% mortality occurred is 20 mg./kg.

3. The cumulative subcutaneous $L.D._{50}$ of pterygospermin is about 50 mg. and acute subcutaneous $L.D._{50}$ 300 mg./kg.

4. Experiments show that pterygospermin like allicin is stable in presence of blood and gastric juice but unstable in presence of pancreatic juice. Experiments to find out the efficiency of pterygospermin, against bacterial infections of laboratory animals is in progress.

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References

1. George, M., *et al.* .. *J. Sci. R Ind. Research*, 1947, **6B**, 43.
2. Rao, R. R., and George, M. Under publication.
3. ——— *et al.* .. *J. Sc. Ind. Res.*, 1946, **5 B**, 31.
4. Maney and Kuever .. *J. Amer. Pherm. Assn.*, 1941, **30**, 276.

PREPARATION, PURIFICATION AND COMPOSITION OF PECTINS FROM INDIAN FRUITS AND VEGETABLES

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Considerable attention has been devoted in recent years to the isolation of pectins from plant materials indigenous to India and the elucidation of their chemical composition. Singh and Dutt (1941) studied the distribution of pectin in a variety of fruits like wood apple, *bel*, guava, etc., and the physico-chemical conditions affecting the formation of sugar-pectin-acid jellies from these fruit juices. Damodaran and Rangachari (1945) have estimated the pectin content of nearly twenty fruits and vegetables and have attempted to prepare pectins from these sources in a pure condition. Savur and Srinivasan (1946) examined various fruits and vegetables for their pectin content and found several of them to be good sources of high grade pectin. Trehan and Ahmed (1947) have given the pectin content of several fruits, vegetables and nuts and have drawn attention to the fact that the ash content of crude pectins obtained from these sources can be reduced to a considerable extent by repeated precipitation with alcohol.

Although the materials investigated by the above authors cover a wide range of fruits and vegetables abundantly available in India, no attempt has, however, been made so far to correlate the pectin contents, chemical composition and jelly forming power of various preparations. The present paper is an attempt in this direction and describes the results obtained in the course of an investigation on the distribution, preparation and purification of pectins from a variety of plant materials and their possible application in jelly making.

Experimental

Preliminary treatment of materials handled

Papaya.—Fruits which had just begun to ripen were chosen. They were cut into small pieces and minced in a meat mincer recovering as much of the juice as possible. The minced pulp was pressed in cloth bags and the juice obtained was mixed with the juice from the mincer. The pressed pulp was washed with some water and the wash water added to the

juice. The collected juice was received in a clean flask, thoroughly mixed, weighed and used for a separate estimation of the pectin content. The sugars, the colouring matter and other soluble impurities were removed by repeated washing and decanting with water. The pulp was soaked in 95% alcohol and left overnight, then pressed free from alcohol, rewashed with water, pressed free of as much water as possible, spread on trays and dried in a current of hot air (50 to 55° C.). The dried material though it retained a part of the moisture was found to preserve well for quite a long period. The dry material was powdered to 100 mesh and kept in air-tight bottles. The material was found to keep well for over one to two years. The treatment for unripe papaya was just the same. The juice, however, was not taken for estimation. Melon fruit, cucumber, guava and plantains were treated in a similar manner.

Jack fruit.—The fruit was separated into the seeds, edible kernel and pericarp. They were separately cut into small bits and dried in a current of warm air. The seeds on drying and powdering yielded a starch-like powder; the kernels gave a flour with a pleasant odour and the pericarp a loose buff coloured powder.

Tamarind and Indian gooseberry.—The materials were dried and ground into powdery consistency without removing the organic acids. Before estimation the organic acids were removed by repeated percolation with cold water. Pectins removed in the percolate were estimated separately. The percolated material was treated as for papaya.

Locust bean and tamarind seed.—These had hard outer coats which required the following processing for complete removal. The seeds were soaked in water and kept for forty-eight hours. A layer of toluene on the water surface acted as a preservative. The coats could be easily peeled off after this soaking. The kernels were then cut into pieces and dried as before.

Extraction of pectic substances

5 to 10 gm. of the dry powder were weighed and ground with 5 gm. of chemically treated sand after slightly moistening the mixture. Three such lots were taken in 500 c.c. conical flasks. 350 c.c. lots of distilled water, 0·5% oxalic acid solution and 0·5% ammonium oxalate solutions were added to the first, second and third flasks respectively. The flasks were plugged with cotton and put on a water-bath maintained at 80 to 90° C. Extraction was carried on for 18 hours continuously. The extract was first filtered through two layers of muslin cloth and subsequently through fluted filter-papers. The clear filtrate was made up to volume and preserved under

toluene. The same extract was used for all the four following methods of estimation:

1. *Alcohol precipitation method.*—Aliquot portions of the extracts were taken in beakers and concentrated to a small bulk on a water-bath. The oxalic acid extracts were neutralised with 0·1% alkali prior to concentration. Three and half times by volume of 95% alcohol which had been previously acidified with a few drops of hydrochloric acid to make the final concentration of the acid to 0·1 N were added to the concentrate in a thin stream with constant stirring. The voluminous precipitate that separated out was thoroughly mixed and left overnight for settling. The precipitate was carefully transferred to a tared filter-paper, washed successively with hot 60%, 75% and 95% alcohol till free from salts and finally with a few drops of absolute alcohol followed by ether. The filter-paper and contents were dried to constant weight at 105° C. and the weight taken after which the precipitate with the filter-paper was incinerated in a silica crucible and heated to dull redness in a muffle furnace at 600 to 750° C. for about four hours and the ash weighed. The weight of the ash was subtracted from the original weight of the precipitate and the pectin content expressed as percentage of ash-free alcohol precipitate.

2. *Estimation as calcium pectate* following the method devised by Carre and Haynes (1922, 1926) and modified by Nanji and Norman (1928). After noting down the weight of calcium pectate in each case, the calcium content of the precipitate was estimated by incinerating the precipitate and estimating the calcium in the ash.

3. *Estimation of pectin as pectic acid* (modified Wichmann's method) (Wichmann, 1922). The ash content of the pectic acid precipitates were subtracted from the original weights of the precipitates.

4. *Titration method* (Ahmann and Hooker, 1926). The temperature used for saponification was 55° C. and the time twelve hours.

A. Pectin Content of Some Common Fruits and Vegetables

In Table I are given the moisture content and calcium pectate yields by differential extraction of nearly a dozen plant materials.

The results show that papaya, jack fruit, sweet melon, ash pumpkin and Indian gooseberry are very rich sources of pectin. The locust bean imported from the Mediterranean countries is mainly used in the textile industry as a sizing material. It is evident that its application as a sizer is virtually dependent on its pectin content. Although it gave very high

TABLE I *Moisture and pectin content of some common vegetables and fruits*

Name of Material	Botanical Name	Moisture %	Method and the extractants used	Method of estimation				
				Alcohol ppte.	Calcium pectate	Calcium % in cal. pect.	Titration	Pectic acid
Ripe papaya fruit ..	*Carica papaya*	92	A— Water	2·13	1·40	7·44	1·49	1·32
			B—0·5% Oxalic acid	28·67	20·58	7·48	21·19	15·21
			C—0·5% Ammon. oxalate	42·92	34·67	7·56	35·53	21·62
Unripe papaya ..	*do*	85	A	13·10	10·02	7·39	10·21	8·93
			B	42·64	30·78	7·47	31·70	27·52
			C	48·75	33·57	7·38	34·19	28·35
Jack fruit ..	*Artocarpus integrifolia*	Kernel 70	A	15·76	2·02	7·47	2·08	1·56
			B	23·04	2·74	7·53	2·93	2·04
			C	23·81	6·19	7·53	6·31	4·97
		Seeds 59	A	12·22	2·01	7·43	2·03	1·25
			B	13·34	2·85	7·59	2·99	1·97
			C	15·47	3·95	7·49	5·10	3·85
		Pericarp 83	A	11·48	6·57	7·51	6·96	5·79
			B	18·45	13·03	7·53	13·42	12·07
			C	23·01	13·29	7·53	13·95	12·19
Sweet melon ..	*Citrallus vulgaris*	86·5	A	7·37	4·29	7·49	4·58	3·65
			B	15·76	12·87	7·49	14·32	10·59
			C	23·82	16·39	7·51	17·26	14·97
Tamarind fruit pulp without skin and seed after removal of tartaric acid by cold percolation	*Tamarindus indica*	72	A	2·56	0·46	7·53	0·48	0·32
			B	6·48	0·82	7·51	0·89	0·67
			C	7·87	1·03	7·47	1·12	0·92
Immature tamarind pulp after removal of organic acid by repeated cold percolation	*do*	67	A	3·14	0·27	7·48	0·27	0·23
			B	6·27	1·01	7·43	1·12	0·97
			C	6·93	1·13	7·51	1·27	1·07

Indian gooseberry whole fruit after removal of acid by cold percolation	*Phyllanthus emblica*	79	A	4·34	1·12	7·42	1·14	0·80
			B	7·52	6·37	7·46	6·56	4·51
			C	9·32	6·52	7·49	6·85	4·66
Plantains ..	*Musa paradisica*	88	A	2·85	1·25	7·51	1·37	1·01
			B	7·61	3·52	7·47	3·81	3·21
			C	12·39	4·21	7·42	4·71	3·92
Guava ..	*Psidium guava*	71	A	6·79	4·21	7·43	4·32	4·03
			B	10·33	6·03	7·43	6·14	5·89
			C	13·47	6·74	7·48	6·97	6·13
Sweet pumpkin ..	*Cucurbita pepo*	87	A	12·35	2·17	7·43	2·21	1·69
			B	25·93	20·84	7·43	21·88	16·67
			C	26·55	21·43	7·46	22·07	16·73
Cucumber ..	*Cucumus sativus*	95	A	7·65	1·74	7·49	1·92	1·41
			B	18·37	15·65	7·48	15·81	15·12
			C	23·65	18·79	7·48	18·92	18·23
Ash pumpkin ..	*Lagenalia vulgaris*	93	A	9·17	7·75	7·51	7·91	6·40
			B	30·13	24·52	7·52	28·50	19·93
			C	32·51	28·02	7·51	28·86	23·54
Lemons after removal of acids by repeated cold percolation	*Citrus medica*	85	A	18·12	13·17	7·49	13·34	12·91
			B	24·32	18·21	7·48	18·72	17·93
			C	28·37	18·87	7·49	19·03	18·31
Oranges ..	*Citrus nobilis*	87	A	10·76	8·34	7·51	8·72	8·02
			B	14·48	11·73	7·51	11·94	11·27
			C	21·43	12·91	7·50	13·12	12·64
Tamarind seed ..	*Tamarindus indica*	13	A	10·76	0·00	..	..	..
			B	42·31	0·00	..	..	..
			C	58·14	0·00	..	..	..
Locust bean ..		11	A	40·76	32·93	7·53	33·12	31·21
			B	57·68	53·72	7·51	53·87	53·17
			C	69·73	61·05	7·51	61·21	60·84

yields of pectin the jellies prepared were not comparable to those made from papaya or gooseberry.

In Table II are presented the results of estimation of pectin in certain fruit juices. The juices were concentrated under vacuo to give a slurry of a syrupy consistency. Wherever the juice was rich in acid it was neutralised by adding alkali prior to concentration.

TABLE II

Pectin content of certain fruit juices

Material 100 c.c. of the fresh juice used	Alcohol precipitate	Calcium pectate	Titration	As pectic acid
Papaya juice ..	1·82	0·12	0·17	0·09
Water melon juice ..	2·17	0·29	0·31	0·23
Orange juice ..	1·93	0·09	0·11	0·07
Lemon juice ..	1·97	0·05	0·07	0·03
Cane juice ..	1·24	0·03	0·04	0·02
Tomato juice ..	1·76	0·13	0·13	0·07

B. PREPARATION OF PECTINS

Preparation of pectins from the fresh fruits or vegetables was found to be rather difficult since no clear extract could be obtained. The materials were hence subjected to a preliminary treatment similar to the one already described for the estimation of pectins.

Extraction.—The extractants used were (i) N/50 sulphuric acid and (ii) 0·5% ammonium oxalate. The materials were taken in flasks and soaked with the extractants and heated on a water-bath at 80 to 90° C. for six hours. The extraction was repeated twice or thrice. The extracts were pressed through cloth while hot.

Preliminary purification of the extracts.—When the materials were used for the extraction after the preliminary drying process clear filtrates could be obtained in all cases. Refiltration was carried out whenever necessary after treatment with supercel. Shaking up with one part of charcoal per 1,000 parts of the extract and filtration cleared the colour in almost all cases and gave a toning effect to the extracts. Clarification of the cloudy extracts obtained from fresh fruits and vegetables was effected by pressure filtration or centrifuging. In the case of fresh and immature tamarinds the extract was more or less a milky emulsion which did not lend itself either to centrifuging or filtration.

Concentration of extracts.—Concentration of the clear extract was effected by spreading it on shallow trays and exposing to a current of warm

air (55° C.) for a period of eight to twelve hours. After concentration to a syrupy consistency the extract was once again filtered.

Precipitation with alcohol.—The concentrated extract was kept in an open vessel of two to three litres capacity and alcohol added in a thin regulated stream keeping the solution constantly stirred. A few drops of hydrochloric acid were added to the alcohol prior to mixing with the pectin concentrate. The flocculent particles of pectin precipitated were allowed to settle overnight and first filtered through cloth and subsequently transferred to a Buchner and washed repeatedly with warm (60° C.) 65% alcohol and finally with warm (55° C.) absolute alcohol followed by ether. The dry crisp powder that remained on the Buchner was pressed between folds of filter-paper, spread on shallow basins and dried in a current of warm air. The drying was found to leave the pectin more or less crisp when it could be powdered to a uniform consistency.

The yield, moisture content, ash and calcium pectate numbers of pectins prepared from a number of fruits and vegetables are given in Table III.

TABLE III

Yield of pectins from certain fruits and vegetables

Name of material	Extractants used	Yield %	Moisture %	Ash %	Calcium pectate number
Ripe papaya	1. N/50 H_2SO_4	25	13·9	2·16	59·55
	2. 0·5% ammon. oxalate	36	13·36	1·98	67·75
Unripe papaya	1. ..	28	7·50	3·61	50·65
	2. ..	39	15·66	2·83	56·45
Jack fruit seeds	1. ..	21	8·7	3·64	20·64
	2. ..	25	9·37	4·49	24·72
Jack fruit kernel	1. ..	23	6·9	2·31	21·85
	2. ..	28	10·0	2·97	25·39
Jack fruit pericarp	1. ..	25	12·20	2·61	26·12
	2. ..	30	12·54	2·92	37·03
Sweet melon	1. ..	20	2·41	2·01	73·14
	2. ..	27	2·65	2·12	76·87
Sweet pumpkin	1. ..	22	3·16	2·27	49·78
	2. ..	29	4·71	2·31	56·64
Ash pumpkin	1. ..	17	3·57	2·10	74·38
	2. ..	21	3·69	1·48	78·57
Cucumber	1. ..	14	2·73	1·87	32·16
	2. ..	17	3·12	2·12	36·72
Tamarind	1. ..	11	15·07	1·98	9·81
	2. ..	13	8·99	2·17	9·92
Indian gooseberry	1. ..	22	11·24	1·37	31·27
	2. ..	27	10·57	1·89	32·64
Guava	1. ..	14	3·87	3·17	16·48
	2. ..	15	3·92	3·83	18·32

C. Composition of the Pectins Prepared

Furfural was estimated by the volumetric method of Powel and Whittaker (1924).

Uronic anhydride was determined by the method devised by Dickson, *et al.* (1930). From the yield of CO_2 obtained by decarboxylation, the anhydride was calculated according to Norris and Resch (1935). Methoxyl value was estimated by the micro method of Nanji and Norman (1926).

The above three analytical constants for the different pectin preparations are embodied in Table IV.

TABLE IV

Composition of the pectins prepared

Name of material	Extractant used	Uronic anhydride $CO_2 \times 4$	Furfural yield %	Methoxyl value %
Papaya ripe ..	1. Acid ext.	59·44	23·27	5·87
	2. Ammon. ox.	64·28	24·12	6·23
Papaya unripe ..	1. ..	46·04	15·69	4·36
	2. ..	51·84	18·40	4·82
Jack fruit seeds ..	1. ..	16·72	5·18	1·98
	2. ..	19·48	5·75	2·06
Jack fruit kernel ..	1. ..	18·08	6·39	2·87
	2. ..	21·68	6·75	2·92
Jack fruit pericarp ..	1. ..	22·05	8·42	3·16
	2. ..	28·73	10·64	3·89
Sweet melon ..	1. ..	57·16	20·21	5·12
	2. ..	60·88	19·83	5·87
Pumpkin ..	1. ..	38·92	13·23	3·13
	2. ..	45·32	14·11	3·97
Ash pumpkin ..	1. ..	55·56	19·71	5·12
	2. ..	58·48	20·72	5·39
Cucumber ..	1. ..	23·88	7·76	2·87
	2. ..	27·44	8·92	3·12
Tamarind ..	1. ..	9·00	3·05	2·06
	2. ..	8·08	2·74	2·12
Indian Gooseberry ..	1. ..	26·32	9·28	3·87
	2. ..	27·48	9·74	4·16
Guava ..	1. ..	9·48	3·87	0·97
	2. ..	9·78	4·12	1·27

D. Preparation of Sugar-Acid Jellies

Method of jelly making.—The pectin was dispersed in water and extracted on a water-bath and filtered. Known amounts of sugar and acid were boiled with the pectin solution in weighed beakers and concentrated to the jelly forming point. The mixture darkened in colour and tended to boil over which was carefully avoided by regulating the flame.

Sheeting test on the jellies were carried out according to the method suggested by Tarr and Baker (1924). Jelly strength and synerisis temperature were determined according to the methods developed by Singh and Dutt (1941).

The jelly strength, synerisis temperature and composition of the various jellies are embodied in Table V.

TABLE V

Composition and characteristics of pectin jellies from various sources

Material used	Extractant used	Jelly* strength in seconds	Synerisis temp. °C.	Acid† in jelly %	Sugar in jelly %	Pectin in jelly %
Papaya ripe ..	1. N/50 H_2SO_4	93	42	1·42	60·5	0·42
	2. 0·5% Ammon. ox.	97	47	1·38	59·3	0·47
Papaya unripe ..	1. ..	67	34	1·43	62·8	0·48
	2. ..	81	39	1·28	63·2	0·37
Jack fruit seeds ..	1. ..	22	27	1·12	60·8	0·23
	2. ..	23	27	1·14	60·2	0·27
Jack fruit kernel ..	1. ..	82	36	1·03	58·6	0·38
	2. ..	89	39	1·09	59·2	0·47
Jack fruit pericarp ..	1. ..	31	28	0·92	62·7	0·43
	2. ..	33	26	0·85	64·3	0·51
Sweet melon ..	1. ..	68	47	0·87	60·8	0·38
	2. ..	78	51	1·02	59·7	0·49
Sweet pumpkin ..	1. ..	42	38	0·91	61·7	0·31
	2. ..	51	41	0·98	60·8	0·52
Ash pumpkin ..	1. ..	93	49	1·32	63·8	0·28
	2. ..	97	56	1·17	60·2	0·41
Cucumber ..	1. ..	32	25	1·32	62·3	0·32
	2. ..	26	27	1·28	61·8	0·58
Tamarind ..	1. ..	53	42	1·02	60·8	0·48
	2. ..	64	46	1·13	59·8	0·52
Indian gooseberry ..	1. ..	88	48	1·21	58·4	0·43
	2. ..	102	51	1·08	59·6	0·58
Guava ..	1. ..	108	58	1·98	58·2	0·46
	2 ..	118	63	2·01	56·8	0·58

* The ideal jelly required 90 to 100 seconds to pass through a fixed length of the viscometer at room temperature.

† The acidity in each case is expressed as % lactic acid.

E. ATTEMPTS AT PURIFICATION OF THE PECTIN PREPARATIONS

The following methods were employed to effect partial purification of the crude pectin preparations.

(i) *Dialysis.*—5% suspensions of pectins were dialysed against 0·05 N hydrochloric acid in cellophane bags over three-day periods in the cold room using toluene as a preservative. The dialysed solution was concentrated to a small bulk and reprecipitated with alcohol.

(ii) *Treatment with bromine water.*—The method used was essentially similar to that developed by Nanji and Chinoy (1934).

(iii) *Repeated precipitation with alcohol.*—The crude pectins obtained were dispersed in water and precipitated with three and half volumes of alcohol. The precipitates were redissolved in water and reprecipitated with alcohol. The operation was repeated four times.

The calcium pectate number, ash content, uronic anhydride and methoxyl values of the crude and purified preparations obtained by the above methods are embodied in Tables VI, VII and VIII.

TABLE VI

Effect of dialysis of pectins

Material	Treatment	Ash %	Calcium pectate %	Uronic anhydride %
Papaya ripe—				
Acid extraction ..	Before dialysis	2·16	59·55	59·44
	After dialysis	0·98	63·87	62·72
Ammon. oxalate ..	Before dialysis	1·98	67·75	64·28
	After dialysis	0·72	68·32	66·17
Jack fruit kernel—				
Acid extraction ..	Before dialysis	2·31	21·85	18·08
	After dialysis	1·03	22·11	19·31
Ammon. oxalate ..	Before dialysis	2·97	25·39	21·68
	After dialysis	1·21	25·62	23·12
Sweet melon—				
Acid extraction ..	Before dialysis	2·01	73·14	57·16
	After dialysis	1·12	73·38	60·27
Ammon. oxalate ..	Before dialysis	2·12	76·87	60·88
	After dialysis	0·98	77·21	62·13
Indian gooseberry—				
Acid extraction ..	Before dialysis	1·37	31·27	26·32
	After dialysis	0·62	32·08	26·81
Ammon. oxalate ..	Before dialysis	1·89	32·64	27·48
	After dialysis	0·72	33·21	27·51
Guava—				
Acid extraction ..	Before dialysis	3·17	16·48	9·48
	After dialysis	1·21	17·32	9·76
Ammon. oxalate ..	Before dialysis	3·83	18·32	9·78
	After dialysis	1·41	19·17	9·97

TABLE VII

Purification of pectins with bromine water

Material	Papaya ripe		Jack fruit kernel		Sweet melon		Indian gooseberry		Guava	
	Acid extract	Ammon oxalate	Acid extract	Ammon oxalate	Acid extract	Ammon oxalate	Acid extract	Ammon oxalate	Acid extract	Ammon oxalate
Uronic anhydride										
Before treatment	59·44	64·28	18·08	21·68	57·16	60·88	26·32	27·48	9·48	9·78
After treatment	67·42	79·13	22·13	26·34	68·21	71·97	34·41	36·12	12·13	13·08
Calcium pectate number										
Before treatment	59·55	67·75	21·85	25·39	73·14	76·87	31·27	32·64	16·48	18·32
After treatment	68·32	78·32	27·32	34·43	81·37	84·13	38·14	39·34	17·14	19·47

TABLE VIII

Purification by repeated precipitation with alcohol

Material	Ash content	Calcium pectate	Uronic anhydride	Methoxyl value
Papaya ripe				
Ammon. oxalate extraction—				
Crude pectin ..	1·98	67·75	64·28	6·23
First precipitation ..	1·12	68·47	64·43	6·41
Second ,, ..	0·79	72·34	65·87	6·73
Third ,, ..	0·45	73·47	65·98	6·91
Fourth ,, ..	0·27	74·28	66·34	7·08
Sweet melon				
Ammon. oxalate extraction—				
Crude pectin ..	2·12	76·87	60·88	5·87
First precipitation ..	1·72	77·14	61·14	5·92
Second ,, ..	1·03	77·89	61·47	6·04
Third ,, ..	0·52	83·47	63·63	6·21
Fourth ,, ..	0·29	85·32	64·17	6·29
Indian gooseberry				
Ammon. oxalate extraction—				
Crude pectin ..	2·89	32·64	27·48	4·16
First precipitation ..	1·04	34·82	28·12	4·21
Second ,, ..	0·76	37·41	29·34	4·29
Third ,, ..	0·43	39·61	30·14	4·87
Fourth ,, ..	0·21	43·82	32·37	4·92

DISCUSSION

The complex nature of the pectic substances has been known since a long time. It is now fairly well established that the occurrence of the individual pectic substances varies from plant to plant. To standardise the conditions for the preparation of pectins it is of importance to study the distribution of the individual pectic substances. The chief difficulty encountered is in obtaining a completely uniform extraction on account of the inability of the extracting agent to penetrate properly into all the cells of the tissue and its failure to reach and dissolve out the insoluble pectic substances intimately connected with the cell wall either as an incrusting agent or in the form of the middle lamella (Nanji and Norman, 1928). In our studies we have developed methods for the preliminary treatment of materials depending on their individual characteristics. Thus, in the case of fruits containing the juices the latter were quantitatively pressed out and separately estimated for pectin. Most of the soluble materials had been washed away both with water and alcohol which ensured the yield of colourless products during extraction. After the removal of alcohol extractables the material was minced up and dried in a current of warm air and powdered to a fine state of division. Drying at 95° C. as suggested by Link and Tottingham (1923) was found to result in considerable caramelization.

Papaya, guava, pumpkin and jack fruit have been observed by us to contain more pectin than reported by Damodaran and Rangachari (*loc. cit.*). This is evidently due to the fact that the latter authors have used only N/75 hydrochloric acid for the extraction. A perusal of Table I will reveal the necessity of adopting the differential extraction method to get a correct estimate of the pectin content of any material.

A noteworthy feature of the method of preparation of pectins as developed by us is the method of concentration of the aqueous or acid extracts of the pectins. In usual practice the concentration is effected by vacuum evaporation. We have found, however, that mere exposure to a current of warm air is sufficient to bring down the volume of extract to a consistency when it can be precipitated with the minimum amount of alcohol.

We have used three methods of purification of the crude pectins to remove the ash and organic impurities. Dialysis of the preparation against dilute acid removed as much as 60 to 70% of the mineral impurities but resulted in a loss of about 25% of the pectin content during reprecipitation. Treatment with bromine water resulted in an overall increase of 20% of the pectin content. Evidently bromination must be resulting in an oxidation of the organic matter. In the alcohol precipitation method there was a 25% reduction in the mineral matter. A judicious combination of bromination and repeated precipitation with alcohol will in practice work out to be the ideal method of purification.

It would appear from our results that mere presence of pectin in any plant material does not confer upon it the important property of forming jellies in presence of sugar and acid. Although no direct correlation has been observed between either the uronic anhydride content or methoxyl values of the pectins and their jellying power when crude pectins are used, it is likely that these factors may be intrinsically involved in jelly formation.

The results obtained by us indicate the wide scope offered by our indigenous plant materials for the preparation of high grade pectins. Almost all the materials referred to are abundantly available in India and if only there is a proper organised effort to collect these materials in a central place they can be conveniently used for the preparation of pectin as such or jams, jellies and marmalades.

Summary

1. The pectin content of a number of Indian fruits and vegetables has been estimated by four different analytical procedures using a differential extraction method.

2. The conditions for the preparation of pectins from these sources have been studied.

3. The structural constants like calcium pectate number, uronic anhydride, furfural and methoxyl values have been estimated.

4. Jellies have been made using the pectin preparations and their strengths have been estimated.

5. Attempts have been made to purify the crude pectin preparation by three different methods.

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REFERENCES

Ahmann, C. R., and Hooker, H. D. .. *Ind. Eng. Chem.*, 1926, **18**, 412.

Carre, M. H., and Haynes, D. .. *Biochem. J.*, 1922, **16**, 60.

——— .. *Ibid.*, 1946, **20**, 6.

Damodaran, M., and Rangachari, P. N. .. *J. Sci. and Ind. Res.* (*India*), 1945, **4**, 298.

Dickson, A. D., Otterson, H. and Link, K.P. .. *J. Am. Chem. Soc.*, 1930, **52**, 775.

Link, K. P., and Tottingham, N. *U. S. Patent Literature*, 1924.

Nanji, H. R., and Norman, A... *J. Soc. Chem. Ind.*, 1926, **45 T**, 337.

——— .. *Biochem. J.*, 1928, **22**, 596.

Nanji, H. R., and Chinoy, J. J. *Ibid.*, 1934, **28**, 456.

Norris, F. W., and Resch, C. E. *Biochem. J.*, 1935, **29**, 1590.

Powell, A., and Whittaker, T. .. *J. Soc. Chem. Ind.*, 1924, **43 T**, 35.

Savur, G. R., and Srinivasan, A. *J. Sci. and Ind. Res.* (*India*), 1946, **5**, 41.

Singh, B. N., and Dutt, S. .. *Ind. J. Agr. Sci.*, 1941, **11**, 1006.

Tarr, A., and Baker, L. .. *Delaw. Univ. Agr. Bull.*, 1924, **134**.

Trehan, Y. N., and Ahmed, B... *J. Sci. and Ind. Res.* (*India*), 1947, **5**, 16.

Wichmann, A. .. *J. Ass. Off. Agr. Chem.*, 1922, **6**, 34.

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CROP SURVEYS IN BIHAR

I. Studies in the Estimation of Acre-Yield of Sugarcane

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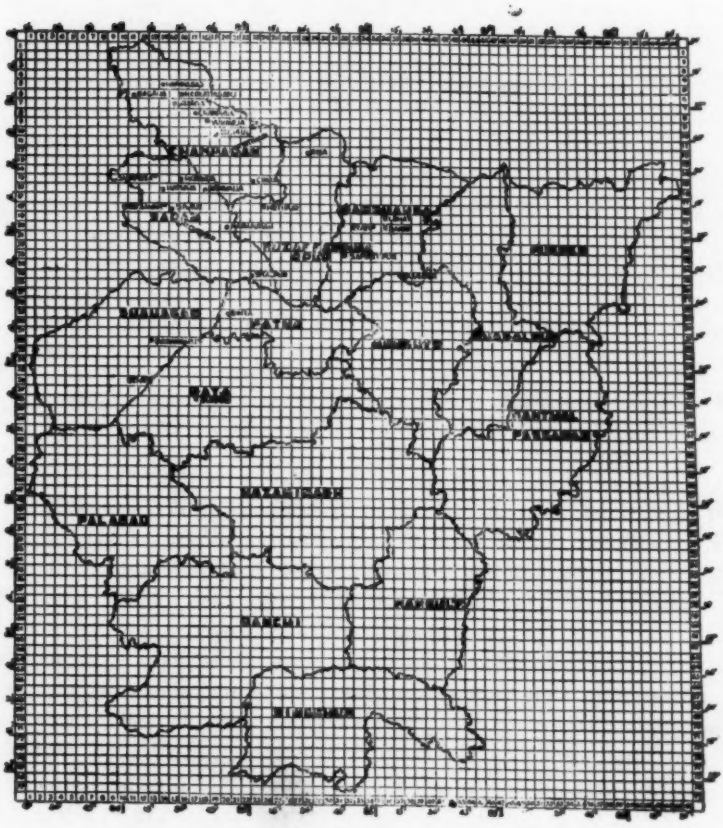


Squared Map of Bihar. 5 × 5 Miles

Section A: General Review

1. *Introduction.*—Sugarcane is the major cash crop of the cultivators in Bihar. With the introducti on and wide spread of Co 313 in North Bihar

and Co 331 in South Bihar after the serious " red rot " epidemic suffered by Co 210 and Co 213, it became necessary to evaluate the yield potentials of the new varieties under different sets of environments. With this aim in view, a planned sample survey on sugarcane was started in 1942–43 (Khanna, 1943) at the initiative and guidance of the Central Sugarcane Research Station; and in view of the encouraging results obtained, this survey was repeated during the subsequent seasons also (Khanna, 1944, 1945), the programme for 1942–43, 1943–44 and 1944–45 being substantially the same. This note gives a brief account of the results obtained during the survey of these three seasons.

The principle of random sampling in crop cutting work was used for the first time in India by Hubback (1927) in his work on paddy in Bihar and Orissa during the years 1923 to 1926. In recent years the Calcutta School of Statisticians under Professor Mahalnobis (1944) has made a distinct advance in the theory of crop cutting experiments, which has been extensively used on various crops in Bengal and elsewhere. Panse and Kalamkar (1944) and Sukhatme (1946) have begun of late similar crop cutting experiments on various crops and are furnishing valuable material for further investigation. The work on sugarcane reported herein was started in Bihar during 1942–43 and may be claimed to be first of its kind in the country so far as this crop is concerned.

2. *Programme.*—The programme (Appendix I) of the survey is given in brief as follows:

The map of Bihar given on p. 169 was divided into squares of 5 miles × 5 miles by drawing lines parallel to North-South and East-West, keeping 'Pusa' as the centre of one of the squares. Villages were selected at random in each square falling within the reserved area and in each village, random selection was made of 4 sugarcane fields. The ultimate sample unit for the crop cutting experiment was a plot of size 32 yds. × 32 yds. marked out at random in the field. The harvest of such a plot was made available in 4 equal portions for convenience in carting—one cart in general being necessary for carrying the harvest proceeds of a quarter of this plot. The harvested crop (stripped canes only) was weighed at the nearest weigh-bridge of the factory concerned, and the yields recorded in the weighment form provided for the purpose. This form along with the forms of village history and field history may be seen at Appendix II.

3. *Acre-yields in general.*—Lists of villages that were supplied to the field staff were drawn, giving as far as possible a proportionate representation to the spread of sugarcane cultivation in each reserved area. But due to some reason or other, all the villages could not be surveyed. Table I

TABLE I

Showing the number of villages and fields surveyed per reserved area during the different seasons

Reserved areas	No. of villages			No. of fields		
	1942-43	1943-44	1944-45	1942-43	1943-44	1944-45
1 Bagaha ..	6	7	9	23	28	36
2 Harinagar ..	9	8	9	36	32	36
3 Narkatiaganj ..	9	10	..	36	40	..
4 Parsa ..	2	4	3	8	16	12
5 Chanpatia ..	10	10	12	40	38	47
6 Majhaulia ..	9	15	14	36	60	56
7 Sugauli ..	10	18	..	38	72	..
8 Motihari ..	6	13	9	24	52	31
9 Chakia ..	8	20	8	31	80	28
CHAMPARAN ..	69	105	64	272	418	246
10 Sasamusa ..	10	18	12	40	72	48
11 Hathua ..	10	16	9	40	64	34
12 Harkhua ..	6	9	9	24	36	36
13 New Siwan ..	8	17	17	32	68	68
14 Indian Siwan ..	4	12	10	16	43	40
15 Sidhwalia ..	6	12	7	24	48	28
16 Pachrukhi ..	10	17	13	40	68	50
17 Marhowrah ..	..	10	11	..	40	44
18 Sitalpore ..	8	15	10	30	56	28
SARAN ..	62	126	98	246	495	376
19 Righa ..	..	4	4	..	13	15
20 Japaha ..	9	..	..	36	..	..
21 Motipur ..	6	8	5	24	32	20
MUZAFFARPUR ..	15	12	9	60	45	35
22 Sakri ..	10	9	6	40	36	24
23 Lohat ..	12	7	4	28	32	16
24 Ryam ..	10	9	4	40	36	16
25 Samastipur ..	8	10	6	32	40	22
26 Hassanpur ..	10	10	..	40	40	..
DARBHANGA ..	50	45	20	180	184	78
NORTH BIHAR ..	196	288	191	758	1142	735
27 Bihta ..	4	5	4	16	20	15
28 Buxar ..	5	8	7	20	30	27
29 Bikramganj ..	8	8	8	32	32	32
30 Dehri-on-Sone ..	8	6	8	32	20	28
31 Gararu ..	10	5	5	10	8	7
SOUTH BIHAR ..	35	32	32	110	110	109
ALL BIHAR ..	231	320	223	868	1252	844

would show the number of villages and fields surveyed in respect of each reserved area in the successive seasons.

TABLE II

Showing the acre-yields per reserved area and the drop during the successive seasons

Reserved Areas	Field per acre in maunds			Drop in yield in 1943–44 over 1942–43		Drop in yield in 1944–45 over 1942–43	
	1942–43	1943–44	1944–45	Absolute drop in mds.	% drop in mds.	Absolute drop in mds.	% drop in mds.
1 Bagha	404·22±24·88	368·16±21·57	320·33± 9·46	− 36·06	− 8·92	− 73·89	−18·28
2 Harinagar	494·68±44·27	472·45±32·97	410·54±31·03	− 23·23	− 4·70	− 84·14	−17·01
3 Narkatiaganj	500·09	380·52±32·97	..	−119·57	−23·91	..	..
4 Parsa	695·84	549·87±43·18	434·57±27·72	−145·97	−20·98	−261·24	−37·54
5 Chanpatia	492·02	424·19±44·89	387·36±10·22	− 67·83	−13·79	−104·66	−21·27
6 Majhaulia	645·35±39·62	497·08	501·45±36·47	−148·27	−22·98	−143·90	−22·30
7 Sugauli	683·11±32·59	714·73	..	+ 31·62	+ 4·63	..	..
8 Motihari	551·33±23·93	447·39	443·34±13·43	−103·94	−18·85	−107·99	−19·59
9 Chakia	536·62±44·22	451·61	392·80±25·07	− 85·01	−15·84	−143·82	−26·80
CHAMPARAN ..	539·58±15·61	494·84±55·58	419·86± 7·80	− 44·74	− 8·29	−119·72	−22·19
10 Sasamusa	566·45±23·41	410·96±13·38	387·34± 7·85	−155·49	−27·45	−179·11	−31·62
11 Hathua	418·42±37·27	336·09±10·64	329·70± 7·62	− 82·33	−19·88	− 88·72	−21·20
12 Harkhua	608·67±49·05	464·79±17·83	468·50±25·97	−143·88	−23·64	−140·17	−23·03
13 New Siwan	395·00±17·08	349·04± 7·33	350·80± 7·47	− 45·96	−11·64	− 44·20	−11·19
14 Indian Siwan	480·33	357·93± 8·89	368·24± 8·14	−122·40	−25·48	−112·09	−23·34
15 Pachrukhi	370·13±12·01	355·80± 9·79	386·06±13·45	− 14·33	− 3·87	− 29·11	− 7·45
16 Sidhwalia	396·86±11·78	387·75± 8·51	361·75± 9·79	− 9·11	− 2·30	− 35·11	− 8·85
17 Marhowrah	..	478·83±14·14	486·94±11·49	..	..	..	..
18 Sitalpur	486·24±11·26	445·23±16·74	373·66±21·71	− 41·01	− 8·43	−112·58	−23·15
SARAN ..	459·99± 9·83	392·13± 4·13	389·95± 4·51	− 67·86	−14·75	− 70·04	−15·23
19 Motipur	521·01±27·04	499·86±16·65	400·05±13·15	− 21·15	− 4·06	−120·96	−23·22
20 Riga	564·32	358·02	722·93±34·86	−206·30	−36·56	−158·61	−28·10
MUZAFFARPUR	541·06±31·64	458·88±16·65	530·16±30·13	− 82·18	−15·19	− 10·90	− 2·01
21 Lohat	562·79±35·52	620·98±23·65	613·76±84·62	+ 58·19	+10·34	− 50·97	+ 9·06
22 Sakri	550·81±23·60	536·40±25·80	700·44±30·22	− 14·41	− 2·62	+149·63	+27·17
23 Ryam	403·11±48·91	402·45±27·67	437·53±41·29	− 0·66	− 0·16	+ 34·42	+ 8·54
24 Samastipur	761·12±56·81	739·73±14·90	711·22±13·91	− 21·39	− 2·81	− 49·90	− 6·56
25 Hassanpur	617·08±32·68	528·55±30·74	..	− 88·53	−14·35	..	..
DHARBHANGA	526·80±18·16	567·47±11·26	631·77±20·34	+ 40·63	+ 7·71	+104·97	+19·92
26 Bihta	578·64±65·27	454·63±30·22	425·78±30·22	−124·01	−21·43	−152·86	−26·42
27 Gurarn	484·15	438·37	477·05±53·78	− 45·76	− 9·45	− 7·08	− 1·46
28 Buxar	589·38±31·93	519·31±31·89	503·91±34·81	− 70·07	−11·89	− 85·47	−14·50
29 Bikramganj	360·55±12·68	455·63±53·78	376·74±53·97	+ 94·08	+26·09	+ 16·19	+ 4·49
30 Dehri	672·49±15·56	669·49±76·91	632·59±67·69	− 3·00	− 0·45	− 39·90	− 5·93
SOUTH BIHAR	525·00±13·29	512·00±25·97	487·15±23·84	− 13·00	− 2·48	− 37·85	− 7·21

The acre-yields estimated from the survey during the three seasons are given in Table II. The frequency distribution of the mean yields are furnished below:

Interval	1942–43	1943–44	1944–45
Upto 400 mds. ..	4	8	11
400—450 mds. ..	3	6	6
450—500 mds. ..	6	8	3
500—550 mds. ..	2	4	2
550—600 mds. ..	7	x	x
Above 600 mds. ..	7	4	5
Total ..	29	30	27

The mean yields varied from 360–761 mds. in 1942–43, 336–740 mds. in 1943–44 and 330–723 mds. in 1944–45. It is evident from the above that there is a drop in yield from year to year. The extent of this drop may be well appreciated, if the factories were grouped with regard to yields as follows:

	1942–43	1943–44	1944–45
Below 450 mds. ..	7	14	17
450—500 mds. ..	8	12	5
Above 500 mds. ..	14	4	5
Total ..	29	30	27

The above table shows that the number of reserved areas having low yields increased and the number of high yielding reserved areas decreased in the latter two seasons, the drop in respect of which has been expressed as percentages of the yield in 1942–43 and the figures furnished in cols. (6) and (8) of Table II.

During 1943–44, all reserved areas except Sugauli in Champaran, Lohat in Darbhanga and Bikramganj in South Bihar showed a drop, which was the highest in Riga, being 36·56%. But in 1944–45, Riga recorded a rise in yield over that of 1942–43 by 28·10%. Lohat, Sakri and Ryam in Darbhanga and Bikramganj in South Bihar also gave higher yields during 1944–45 than in 1942–43. The rest of the reserved areas in 1944–45 showed a distinct drop, the highest of which was 37·54% recorded by Parsa.

4. *High and low yielding areas.*—From the yield figures of the three years, it is possible to demarcate the areas of high and low yields in each district. In Champaran,—Pursa, Sugauli and Majhaulia may be classed as the high yielding areas whereas Bagaha, Narkatiaganj and Chanpatia may

be grouped as low yielding areas; Chakia gave medium yields during all the three seasons. In Saran—Harkhua and Marhowrah yielded the highest, whereas Hathua and New Savan gave low yields. Indian Siwan may, however, be taken as a medium yielding area in this district. In Muzaffarpur,—Riga yielded higher than Motipur during 1942–43 and 1944–45. In Darbhanga, Samastipur was decidedly the highest yielding reserved area in all the three seasons while Ryam recorded the lowest yields. Lohat and Sakri gave only medium yields in the district of Darbhanga. In South Bihar, the highest yield was recorded in respect of Dehri and the next highest was in respect of Buxar in all the three seasons. Bikramganj might be taken as a low yielding reserved area in South Bihar.

5. *Effect of manures.*—The field records revealed that majority of plots were manured with cow dung and in a few cases with castor cake or both. Other manures such as amm. sulphate, compost and gypsum were found to be rarely in use. The average yields obtained from different kinds of manures are furnished below:

Average yields with the number of plots under each type of manure

(Average acre-yield in mds.)

Kind of manure	1942–43	1943–44	1944–45
F.Y.M. ..	537 (682)	438 (900)	421 (594)
Castor cake ..	594 (9)	511 (23)	549 (40)
F. Y. M. + Castor cake	649 (44)	466 (105)	407 (64)
Green manure + F.Y.M.	794 (9)	..	..

It is clear from the above table that castor cake gave higher yields than F.Y.M. in all the seasons. In 1942–43, castor cake with F.Y.M. gave higher yields than any of them alone whereas F.Y.M. after green manuring gave the highest yield. During 1943–44 and 1944–45, castor cake in combination with F.Y.M., however, did not show its response to yield as favourably as it did during 1942–43, this being due largely to the unfavourable nature of the monsoon both in the antecedent and the current year of growth.

6. *Effect of ratooning.*—In all the three seasons, the number of ratooned fields formed only a small fraction of the total fields made available in the survey, the percentages being 13·36 in 1942–43, 9·78 in 1943–44 and 13·03 in 1944–45. The acre-yields available from the ratooned fields have been shown in Table III, where also the number of fields available under ratoon crop in each reserved area has been indicated in 'brackets'. Comparing these yields with the overall averages drawn in respect of the reserved areas, it is found that ratoon crop gave lesser yields in almost all the areas except

TABLE III

Showing the average acre yields per reserved area

(Numbers in brackets show the number of ratooned fields)

Reserved Areas	1942–43		1943–44		1944–45	
	Over all Average	Ratoon	Over all Average	Ratoon	Over all Average	Ratoon
1 Bagaha ..	404·22	252·86 (3)	368·16	253·08 (3)	..	..
2 Harinagar ..	494·68	..	..	..	410·54	264·88 (1)
3 Narkatiaganj	500·09	359·21 (13)	380·52	312·75 (13)	..	..
4 Parsa ..	695·84	..	..	..	439·57	357·40 (4)
5 Chanpatia ..	492·02	394·47 (6)	424·19	378·07 (7)	387·36	272·07 (11)
6 Majhaulia ..	645·35	545·66 (10)	497·08	454·32 (10)	501·45	396·18 (12)
7 Sugauli ..	683·11	480·11 (6)	714·73	266·87 (6)	..	..
8 Motihari ..	551·33	288·78 (12)	447·39	241·14 (12)	443·34	384·41 (11)
9 Chakia ..	536·62	390·21 (4)	451·61	339·38 (4)	392·89	332·61 (7)
10 Sasamusa ..	566·45	292·23 (9)	410·96	292·46 (9)	..	..
11 Hathua ..	418·42	328·48 (2)	336·09	208·12 (2)	..	..
12 Harkhua ..	608·67	388·08 (7)	464·79	390·40 (6)	488·50	357·12 (3)
13 New Siwan ..	395·00	..	..	..	350·80	346·47 (1)
14 Indian Siwan	480·33	..	..	..	368·24	319·28 (1)
15 Sidhwalia ..	390·86	..	387·75	223·26 (2½)	361·75	367·47 (4)
16 Pachrukhi ..	370·13	..	..	..	387·06	409·15 (1)
17 Marhowrah .	..	..	..	..	..	..
18 Sitalpore ..	486·24	435·82 (6)	445·23	444·05 (6)	373·66	342·36 (11)
19 Riga ..	564·32	278·86 (1)	358·02	279·07 (1)	..	..
20 Motipur ..	521·01	..	..	..	..	..
21 Sakri ..	550·81	..	..	..	..	..
22 Lohat ..	562·79	368·66 (2)	620·98	368·94 (2)	613·76	297·99 (2)
23 Ryam ..	403·11	319·03 (8)	402·45	319·28 (8)	437·53	401·44 (2)
24 Samastipur ..	761·12	..	..	..	..	..
25 Hassanpur ..	617·08	..	..	..	..	..
26 Bihta ..	578·64	..	454·63	475·37 (4)	..	..
27 Buxar ..	589·38	482·57 (13)	519·31	434·69 (13)	503·91	480·00 (12)
28 Bikramganj ..	360·55	421·17 (14)	455·63	421·44 (14)	376·74	337·06 (27)
29 Dehri ..	672·49	..	..	..	..	..
30 Gurarn ..	484·13	..	..	..	..	..

	1942–43	1943–44	1944–45
% of ratoon crop	13·36	9·78	13·03

in Bikramganj in 1942–43, Bihta in 1943–44 and Sidhwalia and Pachrukhi in 1944–45.

7. *Acre-yields with reference to different environments.*—From the records available in the field history forms, the yields were classified under different soil types, field levels and water-logging conditions with a view to getting an idea of the extent of the yield differences (if any) that might have existed in the cultivators' fields as they were, under such environmental conditions. Though it may not be quite justifiable to attribute the reasons of such differences if they existed at all, to these environments alone, an

average drawn from a reasonably large number of fields under each of these categories might show the trend of the differential response of these environments to yields, as there would exist a fair chance of the other effects getting neutralised in a large sample.

When the reserved areas were examined individually, no consistent trend seemed to have been apparent of the yields under these classes. But when the average yields of North Bihar as a whole were examined, it was noticed that under each of the field levels, non-water-logged plots gave in general higher yields than water-logged plots in all the three years, except only in a few cases. The exceptions were noticed in more cases under low-lying fields, where water-logged plots in heavy and medium soil during 1942–43; in heavy, loamy and clayey soil during 1943–44 and in clayey soil during 1944–45 gave higher yields. It was further noticed that water-logged plots in level fields having medium soil gave the highest yields in 1942–43 and 1943–44. During 1944–45, water-logged plots in level fields and medium soil gave medium yields. This, therefore, showed that water-logged condition with medium soil was favourable to yield in low and level fields.

From an examination of the district averages as well as the averages drawn in respect of North Bihar and South Bihar under the different classes it was clear that medium soil in level or high fields had been highly beneficial to yield.

8. *Effect of the size of sugarcane holding.*—The cultivators were classified according to the size of sugarcane holdings and the out-turn per acre

TABLE IV

Yield per acre (in maunds) according to different sizes of holdings, North Bihar

Size of holding	Year yield per acre																							
	1942–43							1943–44							1944–45									
	Upto				Above 650 mds.	Total	Average yield in mds. per ac.	Upto				Above 650 mds.	Total	Average yield in mds. per ac.	Upto				Above 650 mds.	Total	Average yield in mds. per ac.			
	350 mds.	450 mds.	550 mds.	650 mds.				350 mds.	450 mds.	550 mds.	650 mds.				350 mds.	450 mds.	550 mds.	650 mds.						
Acres																								
Upto 1	60	97	47	46	43	293	471	125	141	84	24	24	398	420	109	111	41	11	23	295	466			
2	25	63	36	27	70	221	498	88	126	54	28	54	350	453	84	83	32	3	20	222	466			
3	16	16	9	11	34	86	536	23	46	16	10	13	108	448	24	35	9	8	9	85	433			
4	5	9	6	16	14	50	550	16	20	11	9	17	73	488	6	13	3	4	3	29	446			
5	3	3	2	3	9	20	560	14	17	13	5	8	57	458	7	9	..	5	1	22	477			
6	2	2	1	3	5	13	554	2	8	4	3	6	23	513	8	3	..	1	..	12	350			
Average 6	4	11	10	15	13	53	542	14	37	18	7	26	102	494	12	17	7	3	3	44	413			
Total	115	201	111	121	188	736	509	282	395	200	86	148	1111	448	252	271	92	35	59	709	413			

obtained under each class. The frequency distribution of the cultivators in the two-way classification has been shown in Tables IV and IV A separately for North and South Bihar. It will be noticed in Table IV that the

TABLE IV (A)

Yield per acre (in maunds) according to different sizes of holdings, South Bihar

Size of holding		Year yield per acre																				
		1942–43							1943–44							1944–45						
		Upto				Above 650 mds.	Total	Average yield in mds. per ac.	Upto				Above 650 mds.	Total	Average yield in mds. per ac.	Upto				Above 650 mds.	Total	Average yield in mds. per ac.
		350 mds.	—450 mds.	—550 mds.	—650 mds.				350 mds.	— 450 mds.	— 550 mds.	— 650 mds.				350 mds.	— 450 mds.	— 550 mds.	— 650 mds.			
Acres																						
Upto	1	9	4	4	11	2	30	477	11	11	1	3	8	34	459	7	4	5	1	3	20	445
	2	4	8	4	11	16	43	563	8	13	4	2	10	37	481	10	7	9	4	6	36	469
	3	..	6	1	2	5	14	543	3	2	1	3	4	13	523	4	3	5	5	11	28	557
	4	1	..	..	5	2	8	588	..	4	2	..	4	10	540	4	1	2	1	2	10	460
	5	..	..	..	..	2	2	700	..	2	..	1	..	3	467	..	1	1	..	..	9	378
	6	4	..	1	..	1	6	400	..	..	1	..	1	2	600	4	3	2	..	..	9	378
Above	6	2	1	2	1	2	8	500	1	3	2	1	5	12	550	..	..	..	1	1	2	650
Total		20	19	12	30	30	111	528	23	35	11	10	32	111	494	29	19	24	12	23	107	482

yields steadily increased up to the size of 5 acres in 1942–43 and upto 4 acres in 1943–44, and 1944–45. In South Bihar also, the same trend is visible in respect of 1942–43 and 1943–44, while during 1944–45, the yield showed increase up to the size of 3 acres instead of upto 4 acres. The trend as shown individually by the three successive seasons therefore goes to suggest that there is an association between the size of holding and the out-turn per acre, smaller holdings giving less yields. The law of ' diminishing return ' may be said to have operated when the size of sugarcane holding increased beyond 5 acres in 1942–43 and 4 acres in 1943–44 and 1944–45. Though the occurrence of a comparatively fewer number of cultivators with higher sizes of holding has somewhat detracted from the conclusive value of the above statement, it was evident none the less that the sugarcane holding between 4–5 acres gave the highest yield.

SECTION B: REQUISITE SAMPLE SIZE

1. *Structure of the variance.*—As the sampling was of the twofold nested type [Yates and Zacopany (1935) and Cochran (1939)] the variance of the mean yield would be given by

$$V(X) = \frac{\Sigma n_i^2}{n^2} \sigma_1^2 + \frac{1}{n} \sigma_2^2, \qquad \text{(i)}$$

TABLE V

Estimates of true variances between villages and between fields in the different reserved areas

1942–43

Reserved areas	No. of villages surveyed	No. of fields surveyed	Variance due to villages (plot basis) σ_1^2	Variance due to fields (plot basis) σ_2^2	Mean yield in mds. per acre ± S.E.	S. E. as %age of mean
Bagaha ..	6	22	91·8414	274·1890	404·22±24·88	6·16
Harinagar ..	9	36	755·0044	130·7691	494·68±44·27	8·95
Narkatiaganj* ..	..	..	..	..	500·09	..
Parsa* ..	..	..	..	..	695·84	..
Chanpatia ..	..	..	..	..	492·02	..
Majhaulia ..	9	36	348·4464	1130·9595	645·35±39·64	6·14
Sugauli ..	10	38	338·2517	489·1013	683·11±32·59	4·77
Motihari ..	6	24	0	614·0373	551·33±23·93	4·34
Chakia ..	8	31	106·1394	2297·5181	536·62±44·22	8·24
CHAMPARAN ..	48	187	307·7852	830·8450	539·58±15·61	2·89†
Sasamusa ..	10	40	111·3170	533·7604	566·45±23·41	4·13
Hathua ..	10	40	495·1703	504·1979	418·42±37·27	8·91
Harkhua ..	6	24	410·6150	936·5113	608·67±49·05	8·06
New Savan ..	8	32	76·0040	111·9961	395·00±17·08	4·32
Indian Siwan* ..	..	..	..	..	480·33	..
Sidhwalia ..	6	24	0	149·2092	396·86±11·78	2·97
Pachrukhi ..	10	40	39·8496	99·6671	370·13±12·01	3·24
Marhowrah ..			Not surveyed	..		
Sitalpore ..	8	32	16·1448	117·1250	486·24±11·26	2·32†
SARAN ..	58	232	166·0588	340·0611	469·99± 9·83	2·14
Riga* ..	..	..	..	..	564·32	..
Motipur ..	6	24	176·0783	200·1597	521·01±29·04	5·57
MUZAFFARPUR ..	..	..	..	..	..	..
Sakri ..	10	40	156·5909	372·2776	550·81±23·60	4·28
Lohat ..	7	28	221·5953	695·9129	562·79±35·52	6·31
Ryam ..	10	40	1053·6682	67·5880	403·11±48·91	12·13
Samastipur ..	8	32	1065·3505	353·0710	761·12±56·81	7·46
Hassanpur ..	10	40	207·9801	866·3927	617·08±32·68	5·30
DARBHANGA ..	45	180	550·4003	461·3009	526·80±18·16	3·45†
Bihta ..	4	16	439·7162	1290·4010	578·64±65·27	11·28
Buxar ..	5	20	181·7056	184·2990	589·38±31·93	5·42
Bikramganj ..	8	32	0	229·8177	360·55±12·68	3·52
Dehri ..	8	32	49·3679	148·0586	672·49±15·56	2·31
Gararu* ..	..	..	..	..	484·13	..

* S.E. could not be calculated in these reserved areas.

† S.E. has been calculated after eliminating variations due to reserved areas.

where n_i is the number of fields in the ith village $\Sigma_{n_i} = n$ (total number of fields) and σ_1^2 and σ_2^2 are respectively the estimates of the true variances between villages and between fields. If v_1 be the mean square between

villages and v_2 that between fields, v_2 will be the unbiased estimate of σ_2^2 and v_1 the unbiased estimate of

$$\frac{n-\Sigma n_i^2/n}{t-1}\sigma_1^2+\sigma_2^2, \qquad \text{(ii)}$$

where 't' is the number of villages surveyed. When, however, the second order zones are equally distributed in the first, the variance of the mean yield would reduce to

$$V(X)=\frac{\sigma_1^2}{t}+\frac{\sigma_2^2}{tf}, \qquad \text{(iii)}$$

where 't' is the number of first order zones (villages) and 'f' the number of second order zones (fields) in each of the first.

2. *Contribution to the error variance made by fields.*—It will be seen in formula (iii) that the contributions to the variance of the mean made by villages and fields are $\frac{\sigma_1^2}{t}$ and $\frac{\sigma_2^2}{tf}$ respectively. The proportion of the variation due to fields will be more in magnitude than that due to villages only when $\sigma_2^2 > f\sigma_1^2$. In the present case, however, 'f' is equal to 4, as in most of the reserved areas, 4 fields have been taken per village. It will be noticed that 6 reserved areas in Table V (1942–43), 8 reserved areas in Table VI (1943–44) and 12 reserved areas in Table VII (1944–45) have shown field variations larger than 4 times the village variations. These reserved areas therefore bring out the necessity for the increase in the number of fields per village in reducing the standard errors. It will be noticed however, that in most of the reserved areas, the standard errors are already low. Therefore, increase in the number of fields per village in such areas will not be of much advantage from the practical point of view.

3. *Magnitudes of the standard errors as found in the reserved areas.*—The standard errors expressed as percentages of the mean yields have been set out in col. (7) of Tables V, VI and VII. The percentages vary between 2·31 and 12·13 in 1942–43, between 2·01 and 11·80 in 1943–44 and between 1·96 and 14·33 in 1944–45. The table given below shows the number of reserved areas falling under different ranges of standard errors:

	Upto 5%	Betwen 5% and 7%	Between 7% and 10%	Between 10% and 14·33%
1942–43 ..	10	6	5	2
1943–44 ..	13	5	3	3
1944–45 ..	14	5	4	4

TABLE VI

Estimates of true variances between villages and between fields in the different reserved areas

1943–44

Reserved Areas	No. of villages surveyed	No. of fields surveyed	Variance due to villages (plot basis) σ_1^2	Variance due to fields (plot basis) σ_2^2	Mean yield in mds. per acre ± S E.	S.E. %age of mean
Bagaha ..	7	28	100·8227	180·2073	368·16±21·57	5·86
Harinagar ..	8	32	..	1553·6947	472·45±32·97	6·98
Narkatiaganj ..	10	40	322·2741	655·4100	380·52±32·97	8·66
Parsa ..	4	16	296·0414	150·3396	549·87±43·18	7·85
Chanpatia ..	10	38	856·2941	130·0484	424·19±44·89	10·58
Majhaulia* ..	10	..	..	..	497·08	..
Sugauli* ..	10	..	..	..	714·73	..
Motihari* ..	10	..	..	..	447·39	..
Chakia * ..	10	..	..	..	451·61	..
CHAMPARAN ..	39	154	300·1735	588·1032	494·84±55·58	11·23†
Sasamusa ..	19	76	152·3700	300·0998	410·96±13·38	3·26
Hathua ..	16	64	..	202·3937	336·09±10·64	3·17
Harkhua ..	9	36	61·9065	263·7182	464·79±17·83	3·84
New Savan ..	17	68	28·1116	51·6550	349·04± 7·33	2·10
Indian Siwan ..	11	39	17·6747	71·2499	357·93± 8·89	2·48
Sidhwalia ..	12	48	23·3830	62·9293	387·75± 8·51	2·19
Pachrukhi ..	17	68	57·6779	59·6059	355·80± 9·79	2·75
Marhowrah ..	10	40	53·2038	144·0188	478·83±14·14	2·95
Sitalpore ..	15	56	135·3042	139·5271	445·23±16·74	3·76
SARAN ..	126	495	62·7903	128·9916	392·13± 4·13	1·05†
Riga ..	10	..	..	..	358·02	..
Motipur ..	8	32	99·3995	148·5472	499·86±16·65	3·33
MUZAFFARPUR						
Sakri ..	9	36	94·8810	692·0272	536·40±25·80	4·81
Lohat ..	8	32	42·2082	629·9147	620·98±23·65	3·81
Ryam ..	9	36	272·3840	141·6296	402·45±27·67	6·88
Samastipur ..	10	40	24·8632	298·6792	739·73±14·90	2·01
Hassanpur ..	10	40	322·8146	399·7645	528·55±30·74	5·82
DARBHANGA ..	46	184	154·9461	424·4921	567·47±11·26	1·98†
Bihata ..	5	20	89·8932	456·5708	454·63±30·22	6·65
Buxar ..	8	30	318·6882	712·5582	519·31±37·89	7·50
Bikramganj ..	8	32	888·9035	584·4518	455·63±53·78	11·80
Dehri ..	6	20	1341·6715	321·1016	669·49±76·91	11·49
Gararu* ..	..	..	..	..	438·37	..

* S.E. could not be calculated in these reserved areas.

† S.E. has been calculated after eliminating the variations due to reserved areas.

TABLE VII

Showing estimates of true variances between villages and between fields in the different reserved areas

1944–45

Reserved areas	No.of villages surveyed	No. of fields surveyed	Variance due to villages (plot basis)	Variance due to fields (plot basis)	Mean yield in mds. per acre ± S. E.	S.E. %age of mean
1	2	3	4	5	5	7
Bagaha ..	9	36	6·9054	116·5585	330·33± 9·46	2·86
Harinagar ..	9	36	326·4017	246·5584	410·54±31·03	7·56
Narkatiaganj ..	0		Not surveyed			
Parsa ..	3	12	..	413·4133	434·57±27·72	6·38
Chanpatia ..	12	47	..	220·1414	387·36±10·22	2·64
Majhaulia ..	14	56	647·1009	739·9940	501·45±36·47	7·27
Sugauli ..			Not surveyed			
Motihari ..	9	31	19·3331	182·2173	441·34±13·43	3·03
Chakia ..	6	22	154·0529	50·8500	392·80±25·07	6·38
CHAMPARAN ..	62	240	82·9699	179·2936	419·86± 7·80	1·86†
Sasamusa ..	12	48	4·7918	113·4167	387·34± 7·85	2·03
Hathua ..	9	34	..	88·4975	329·70± 7·62	2·31
Harkhua ..	9	36	159·5541	446·2489	469·50±25·97	5·54
New Siwan ..	17	68	31·2589	45·3811	350·80± 7·47	2·13
Indian Siwan ..	10	40	15·4127	57·1667	368·24± 8·14	2·21
Sidhwalia ..	7	28	18·8582	44·8571	361·75± 9·79	2·71
Pachrukhi ..	13	50	87·0900	93·9347	386·06±13·95	3·61
Marhowrah ..	11	44	44·2974	83·2290	486·94±11·49	2·36
Sitalpur ..	10	28	174·8417	47·6076	373·66±21·79	5·83
SARAN ..	98	376	61·6599	100·0161	389·95± 4·51	1·16†
Riga ..	4	15	139·2909	294·3040	722·93±34·56	4·82
Motipur ..	5	20	30·9727	30·5813	400·05±13·15	3·29
MUZAFFARPUR	9	35	..	1421·5630	530·16±30·13	5·68 †
Sakri ..	6	24	..	980·1178	708·44±30·22	4·27
Ryam ..	4	16	176·0000	516·5200	437·53±41·29	9·44
Lohat ..	4	16	909·7254	1431·8700	613·76±84·62	13·79
Samastipur ..	6	22	20·2203	114·7605	711·22±13·91	1·96
Hassanpur ..			Not surveyed			
DARBHANGA ..	20	78	178·6845	738·9491	631·77±20·34	3·22†
Bihta ..	4	15	114·4999	178·3454	425·78±30·22	7·10
Buxar ..	7	27	143·0719	136·1680	503·91±34·81	6·91
Bikramganj ..	8	32	1033·5920	29·3617	376·74±53·97	14·33
Dehri ..	8	28	..	5735·9400	632·59±67·69	10·70
Gararu ..	5	7	..	905·4008	477·05±53·78	11·27

† S.E. has been calculated after eliminating the variations due to reserved areas.

Taking into consideration all the three seasons, it may be seen in the above table that 50% of the reserved areas have shown standard errors well within 5% of the mean yield, and 22% of the reserved areas have standard errors between 5% and 7%.

4. *Sampling requirements in the areas with high errors.*—From each of the three seasons, 8 reserved areas have been selected where the standard errors have been the highest; and the zone variances in these reserved areas have been subjected to further scrutiny to see the relative importance of the number of villages and fields in the reduction of error. On the basis of an error of 5%, some hypothetical combinations of fields and villages have been computed in Table VIII, with the help of the following formula:

$$t = \frac{f\sigma_1^2 + \sigma_2^2}{f(y \times 0{\cdot}05)^2} \times C^2, \qquad \text{(iv)}$$

where y = yield per acre, 't' = number of villages; f = 6, 4, 2 (number of fields per village) and C is the acreage conversion factor. This table will show that in the reduction of error, increase in the number of villages in the sample is more important than increase in the number of fields. There is practically no difference in the number of villages when the number of fields is increased from 4 to 6. The village variations have indeed been so predominant that in many reserved areas, increase in the number of fields even from 2 to 6 has brought about no substantial reduction in the number of villages.

It has already been mentioned that only those reserved areas have been selected for the purpose of Table VIII, where the standard errors have been the highest. As a matter of fact, all the reserved areas where the errors have been between 7% and 14·33% have been brought under this category. Therefore, the sample size recommended for these reserved areas is expected to be more than sufficient for the other reserved areas where the errors have been already low. Leaving out of consideration the extreme cases (underlined), it may perhaps be said that sample of about 20 villages per reserved area of average size (or 4–5 villages per square of 5 miles × 5 miles) with 4 fields per village would serve the purpose of getting the information on acre-yields within an error of 5 per cent.

It will be interesting to notice that the district of Saran has shown consistently low errors from year to year, whereas some reserved areas in the district of Champaran and South Bihar have in general shown high errors. Harinagar and Ryam occur in the list of high standard errors in all the three seasons.

TABLE VIII

Different combinations of fields and villages on the basis of 5 per cent. standard error

Reserved Areas (S.E.)	σ_1^2 (No. of villages)	σ_2^2 (No. of fields)	Number of villages to reduce the S.E. to 5%		
			For 2 fields per village	For 4 fields per village	For 6 fields per village
		1942–43			
Harinagar .. (8·96)	755·00(9)	130·77(36)	30	29	28
Chakia .. (8·23)	106·14(8)	2297·52(31)	39	21	15
Hathua .. (8·91)	495·17(10)	504·20(40)	38	32	30
Harkhua .. (8·06)	410·62(6)	936·51(24)	21	16	14
Ryam .. (12·13)	1053·67(10)	67·59(40)	60	59	59
Lohat .. (6·31)	221·60(8)	695·91(32)	16	11	10
Samastipur .. (7·46)	1065·35(8)	353·07(32)	19	18	17
Bihta .. (11·28)	439·72(4)	1290·40(16)	29	20	17
		1943–44			
Harinagar .. (6·98)	0 (8)	1553·69(32)	31	16	10
Narkatiaganj .. (8·66)	322·27(10)	655·41(40)	40	30	27
Parsa .. (7·85)	296·04(4)	150·34(16)	11	10	9
Champatia .. (10·58)	856·29(10)	130·04(38)	46	44	44
Ryam .. (6·88)	272·38(9)	141·63(36)	19	17	16
Bikramganj .. (11·80)	888·90(8)	584·45(32)	51	45	42
Dehri .. (11·49)	1341·67(6)	328·10(20)	30	28	28
Buxar .. (7·30)	318·69(8)	712·56(30)	22	16	14
		1944–45			
Harinagar .. (7·56)	326·40(9)	246·56(36)	29	23	21
Majhaulia .. (7·27)	647·16(14)	739·99(56)	40	33	31
Ryam .. (9·44)	272·38(4)	141·63(16)	20	14	12
Lohat .. (13·79)	909·71(4)	1431·87(16)	39	29	27
Bihta .. (7·10)	114·50(4)	178·34(15)	10	8	7
Dehri .. (10·70)	0(8)	5735·94(28)	64	32	21
Guraru .. (11·27)	0(5)	905·40 (7)	18	9	6
Bikramganj .. (14·33)	1033·59(8)	29·36(32)	66	66	65

SUMMARY

Section A.—1. The paper gives a brief account of the crop sample survey that was conducted in Bihar during 1942–43, 1943–44 and 1944–45 with a view to having mainly the information on acre-yields of sugarcane.

2. Of the three seasons under review, the highest acre-yields were obtained during 1942–43 and the yields during the latter two seasons showed a drop. The mean yields varied from 360–761 mds. in 1942–43, 336–740 mds. in 1943–44 and 330–723 mds. in 1944–45.

3. The field records revealed that cow-dung was the most common manure used. Castor cake and castor cake combined with F.Y.M. were

found to have been applied to a few fields only. Castor cake gave higher yields than F.Y.M.

4. The proportions of the ratoon fields to the total number of fields made available in the survey were 13·36% in 1942–43, 9·78% in 1943–44 and 13·03% in 1944–45. The acre-yields recovered from ratoon fields were less than those recovered from plant-crop in almost all the reserved areas.

5. From the classification of the yields under different field levels, soil types and water-logging conditions, it was revealed that non-water-logged plots gave in general higher yields than water-logged plots except in a few types of soil in low-lying fields. Medium soil in level and high fields had been highly beneficial to yield. Medium soil was favourable to yield also in low fields subject to water-logging.

6. The acre-yields showed an association with the size of sugarcane holdings of the cultivators, smaller holdings giving less yields. The tables indicated that the sugarcane holding between 4–5 acres gave the highest yield.

Section B.—Taking into consideration the three seasons together it was found that under the available sample sizes, 50% of the reserved areas gave standard errors well within 5% of the mean yields. The variances due to villages and fields were subjected to scrutiny in cases of the reserved areas where the standard errors were high and it was argued that 20 villages per reserved area of average size with 4 fields per village might in general be sufficient to bring down the standard error in a reserved area to 5% of the mean acre-yield.

ACKNOWLEDGEMENTS

The work was carried out as part of the Sugarcane Research Scheme in Bihar being financed jointly by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks are due. Similarly assistance rendered by the Cane Devlopment Staff who made possible the completion of field work within a specified period during each crushing season and the fullest co-operation by factories in providing facilities in the issue of early challans and in giving preferential treatment at the weigh-bridges, are gratefully acknowledged. Messrs. R. C. Acharya and K. P. Singh assisted at different stages of computational work.

References

1. Cochran, W. G. .. "The use of analysis of variance in enumeration sampling," *Jour. Amer. Stat. Asson.*, 1939, **34**, 492–510.
2. Hubback, J. .. "Sampling for rice yields in Bihar and Orissa," *Imp. Agric. Res. Inst. Bull.*, No. 166, 1927.
3. Khanna, K. L. .. "Yield potential survey," *Rept. Central Sugarcane Res. Sta.*, Pusa, 1942–43, 10, 11.
4. ———— .. "Yield potential survey," *ibid.*, 1943–44, 21–23.
5. ———— .. "Yield potential survey," *ibid.*, 1944–45, 14–16.
6. Mahalnobis, P. C. .. "On large-scale sample surveys," *Roy. Soc. Phil. Trans.*, 1944, **B 231**, 329–451.
7. Neyman, J. .. "On the different aspects of the representative method," *Jour. Roy. Stat. Soc.*, 1934, **97**, 558–625.
8. Panse, V. G., and Kalamkar, R. S. *Curr. Sci.*, 1944, **13**, 120–24.
9. Roy, S. N., and Banerjee, K. S. "On Hierarchical sampling, Hierarchical variances and their connection with other aspects of statistical theory," *Sci. & Cult.*, 1940, **6**, 3, 189.
10. Sukhatme, P. V., and others "Random sampling for estimating rice yield in Kolaba, Bombay," *Proc. Ind. Acad. Sci.*, 1946, **23**, 4, 194–209.
11. Yates, F., and Zacopany, I. "The estimate of efficiency of sampling with special reference to the sampling for yield in cereal experiments," *Jour. Agric. Sci.*, 1938, **25**, 545–77.

APPENDIX I

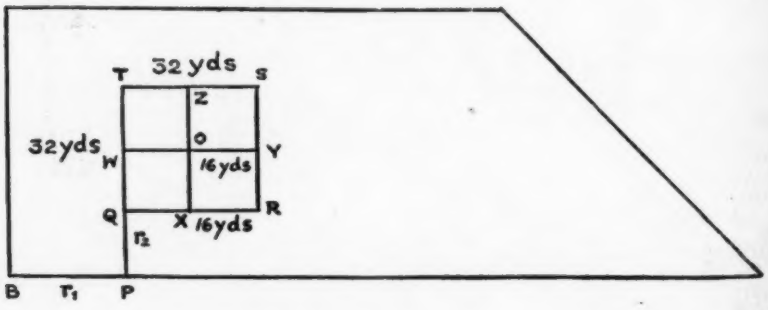
Programme for the Survey of Yield Potential of Sugarcane in Bihar

1. *Selection of villages.*—The map of Bihar will be divided into squares of 5× 5 sq. miles by drawing lines parallel to N-S and E-W with reference to Pusa as the centre of one of the squares and the squares numbered. The number of squares falling on the reserved areas of the sugar factories to be surveyed will be noted with reference to their maps and two villages will be selected at random within each square. This work will be done in the Statistical Laboratory, Central Sugarcane Research Station, Pusa.

The villages selected will be marked on a copy of bigger scale map which may be obtained from the factory concerned.

2. *Selection of holdings and plots.*—A list of holdings in the selected villages will be obtained either from the mill or on going to the village itself. Details about the village conditions will be entered in Form No. I as specified therein. Out of the list of holdings, 4 holdings will be selected by taking lots for yield potential survey. In each holding one field under sugarcane, say, about one acre or more in area, will be selected in a similar manner and field history recorded in Form II. One plot of approximate 1/4th acre (32 yds.× 32 yds.) will then be selected in that field according to the following procedure:

Start from B, the south west corner of the field, and proceed towards east certain number of steps, say r_1 [to be found from Random number tables which will be supplied] and reach a point P. From P go towards north into the field and reach Q, so that PQ= r_2 steps, r_2 being found from the same random number tables as before. Select a field QRST measuring 32 yds. × 32 yds.



The plot QRST will be divided into 4 equal parts of 16 yds. × 16 yds. and harvested and yields recorded in Form II for each by stripping off the canes. This will be arranged with the mill authorities so that proper chalans for cane are issued to the cultivators and cane harvested by them and sent to the weigh-bridge with previous understanding with the clerk over there to weigh the canes and record the weight in the proper form.

3. *Crop density*: 9′× 9′.—One unit of 9′× 9′ say at the corners (Q, X, O, W will be taken in each portion and number of clumps and number of canes for all the clumps will be recorded in Form II.

4. *General.*—It is presumed that the Agricultural Overseer (Cane Development Scheme) with his Kamdars will be able to complete the above work in 10 days.

5. *Supervision.*—The supervision will be entrusted to the Assistant Director of Agriculture (Cane Development Scheme) so far as possible in order to ensure reliable results. It may be suggested that the work (tour) programme will be prepared by the Agricultural Overseer and submitted to the Assistant Director of Agriculture who will then arrange supervision of the work—at least one village for each overseer. The Assistant Director of Agriculture will sign the records that are taken in his presence.

6. All the field records will be sent to the Sugarcane Specialist, Bihar, Pusa, as soon as the work is over.

APPENDIX II

Survey of Yield Potential of Sugarcane

Factory.....

FORM I.—*Village History.*

State 1 *Acre*.....*bigha*.....*Katha*.....*Dhur*.....

Surveyor.....*Date*.....

1. *Village*.....*Thana No*.....*Thana*....*Sq. No.*

2. *Approximate area* (*a*) *Whole village*..... (*b*) *Cultivated all crops* .....

3. *Area under cane per table given below, Source of information.*

	Co 313 B. K.	Co 513 B. K.	Co 313 B. K.	Others B. K.	Others B. K.	Others B. K.	Total B. K.
Plant ..							
Ratoon ..							
Total ..							

4. *Details of holdings which can be selected.*

Sl. No.	Name of cultivator		Area			
	Cultivator	Father	Total holding	Cultivated all crops	Under cane only	Varieties
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

P.S.—Mark with a circle round the Sl. No. for those selected for the survey.

Signatures.....

SURVEY OF YIELD POTENTIAL OF SUGARCANE

Factory.....

FORM II.—*Field History, Crop Density and Yield*

Village.....

Holdings field area Details		I	II	III	IV
1	Cultivator's name ..				
2	Variety (plant or Ratoon) ..				
3	Previous crop ..				
4	Level (High level or low) ..				
5	Flooding, or not ..				
6	Water-logging or not ..				
7	Soil clayey or loam* ..				
8	Manures applied, Kind ..				
	Quantity ..				
9	Cultivations No. of ploughing ..				
	Date of ,, ..				
10	Crop condition † ..				
11	Random No. BP, PQ, B'P', P'Q' ..				
12	Crop density 9' × 9', at Q ..				
	No. of clumps Nos. of cane at V ..				
	Q ..				
	O ..				
13	Yield in mds. seers (1*a*) ..				
	(1*b*) ..				
	(2*a*) ..				
	(2*b*) ..				
14	Total ..				

* Under loam mention light, medium or heavy.

† Under crop condition mention (i) good, (ii) fair, (iii) poor.

DEPARTMENT OF AGRICULTURE, BIHAR

WEIGHMENT FORM.—*Yield Potential Survey*

Factory.....

Name of Cultivator..... *Village*.....

Date	Name of carter	Sl. No. of Gross Wt. sheet	Gross Wt.	Sl. No. of Tare Wt. sheet	Tare Wt.	Net Wt.	Signatute o f Weighment Clerk

N.B.—To be returned along with field forms to C.S.R.S., Pusa.

Checked with factory records and found correct.

Agricultural Overseer.

TONUS AND INHIBITION IN UNSTRIATED MUSCLE

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Received July 23, 1948

In previous papers, it has been shown that there are two kinds of tone, one that requires oxygen and the other that does not (Rao and Singh, 1940; Singh and Singh, 1947 *a*, *b*, 1948). The question arises as to the source of energy for anærobic tone. It is quite possible that the muscle may not be consuming oxygen and yet may be deriving energy from some anærobic mechanism. These points have been investigated in the present research.

The question of inhibition is closely related to that of tonus, as the two being of opposite characters; the study of each will help in the elucidation of the other. It has been shown that there are two kinds of inhibitions in unstriated muscle; during one the oxygen consumption increases and during the other it decreases (Rao and Singh, 1940). This follows from experiments on tonus. If the oxygen consumption decreases during tonic contraction, then during relaxation it should increase and if it increases during contraction it should decrease during inhibition.

Experimental

These experiments were performed on pieces of frog's stomach (Singh, 1939), dog's stomach (Singh, 1940) and fowl's gut (Singh, Singh and Muthana, 1947). If the muscle did not show much tone, then 10 to 20 per cent. of the sodium of the saline was replaced with potassium; this procedure is useful also in giving an idea of the relaxation of the muscle. Resting tone, with a little error, can be considered as possessing zero tension and the tension at any other time can be expressed as percentage of the maximal tension produced by potassium. Other experimental procedures were as in previous papers (Singh and Singh, 1948).

Results

Tonus

Aerobic tone.—This is increased by adding 0·1 per cent. glucose to the saline. This shows that the energy is derived from the production of lactic acid and its subsequent oxidation. If the muscle is poisoned with iodoacetic acid (1 in 20,000), tone still persists, showing that energy is derived

from other sources; in frog's stomach however there may be a marked fall of tonus. In the absence of oxygen the tone rapidly declines. In these respects, therefore, energy for the maintenance of tone is derived from sources similar to those for twitch.

Further analysis shows that the ærobic mechanisms for tone and twitch are not identical. In dog's stomach, as in frog's stomach, there is asphyxial increase of excitability; on reintroduction of oxygen, the response to twitch is depressed as in the frog's stomach, but tone increases (Fig. 1). This

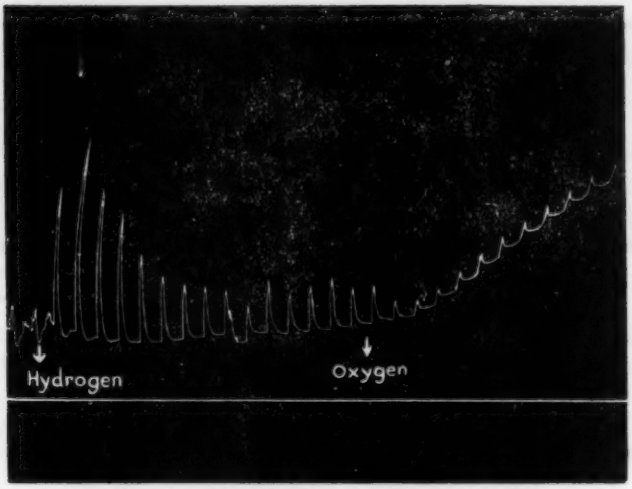

FIG. 1. *Dog's stomach.*—Stimulation by alternating current 12 volts for 12 seconds per minute.

experiment further shows that after asphyxia the muscle becomes more sensitive to oxygen. This is probably due to opening of reserve oxidative channels. This effect of oxygen is similar to that on the chemoreceptors of the carotid body. Watt, Dumke and Comroe (1943) found that the inhalation of pure oxygen by unanæsthetised dogs caused a reduction in breathing but in some animals, the respirations, after an initial reduction, were restored to their original value, although pure oxygen was being breathed.

Results, similar to those described above, are also obtained if the muscle is asphyxiated. The tone as well as the twitch produced by alternating current or acetylcholine decline, but the former declines more rapidly than the latter. The twitch may increase prior to declining (Fig. 2).

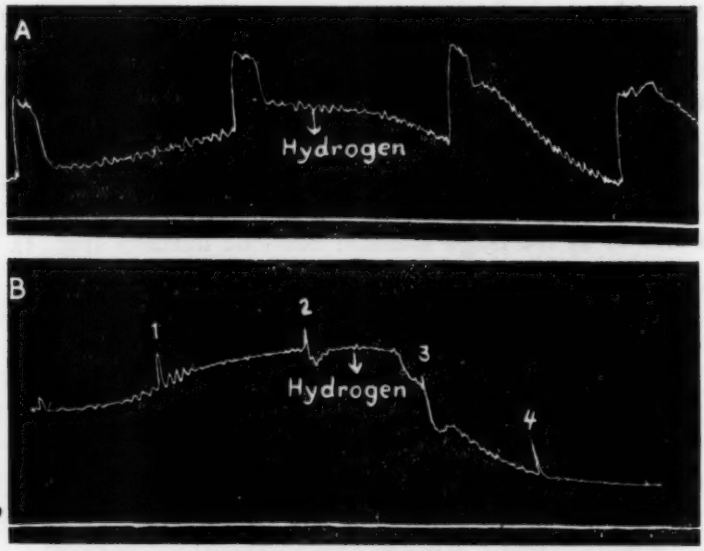

FIG. 2. *Fowl's gut.—A.* Stimulation by acetylcholine, 1 in 10^5. *B.* Stimulation by alternating current at 1, 2, 3 and 4.

Different muscles vary in their sensitivity to oxygen lack; dog's stomach muscle is more resistant than the frog's stomach or the fowl's gut. The latter is very susceptible. In the frog's stomach, there is individual variation. The coronary arteries are known to dilate more than the others as a result of anoxæmia.

Anærobic tone.—In the absence of oxygen, the tone declines to about 50% of its original value in the dog's stomach and almost completely in the fowl's gut. Before declining there may be a phase of increment in the tonus, comparable to the asphyxial increase of excitability to alternating current, potassium and acetylcholine (Fig. 3).

In alkaline solutions, the addition of glucose, 0·1 per cent., causes a sharp recovery which is about 20 to 40 per cent. of the original tension; this can be maintained for several hours (Fig. 4). In fowl's gut, the action of glucose is immediate; contraction is produced as if a stimulant was added. This shows that the action of glucose is on the surface of the cells, and therefore there are enzyme systems on such surfaces which subserve metabolism. This may be important in connexion with the surface action of drugs, hormones and ions which may activate or modify the action of enzymes on such surfaces and so produce their effects. The presence of enzymes on surfaces may account for Clark's active patches on cell surfaces.

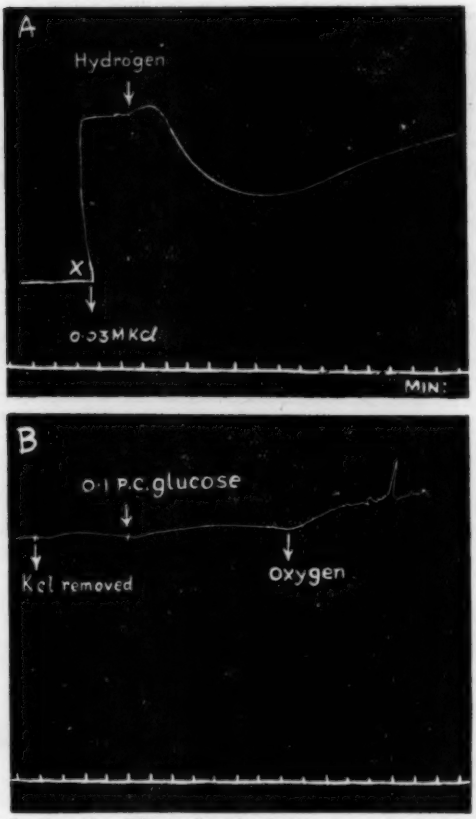

FIG. 3. *Dog's stomach.*—Effect of asphyxia on tone. *B.* Continuation of *A.* At *X*, drum stationary till maximum tension produced.

In the frog's stomach, the action of glucose was not found to be so dramatic (Singh and Singh, 1948). This slower action suggests that glucose is also metabolised inside the muscle fibres. There is also a natural variation in sensitivity of the muscle to glucose just as with other substances.

The sensitivity of unstriated muscle to glucose increases after asphyxia. This is shown by the following experiment on dog's stomach. Addition of glucose in the presence of oxygen hardly causes any increase of tone; asphyxiated muscle may however show a great increase of tone, during which the response to alternating current is depressed (Fig. 5). This shows the difference between the anaerobic metabolisms of tone and twitch. The increased sensitivity to glucose in asphyxia suggests opening of reserve anærobic channels, thus accounting for asphyxial hyperexcitability.

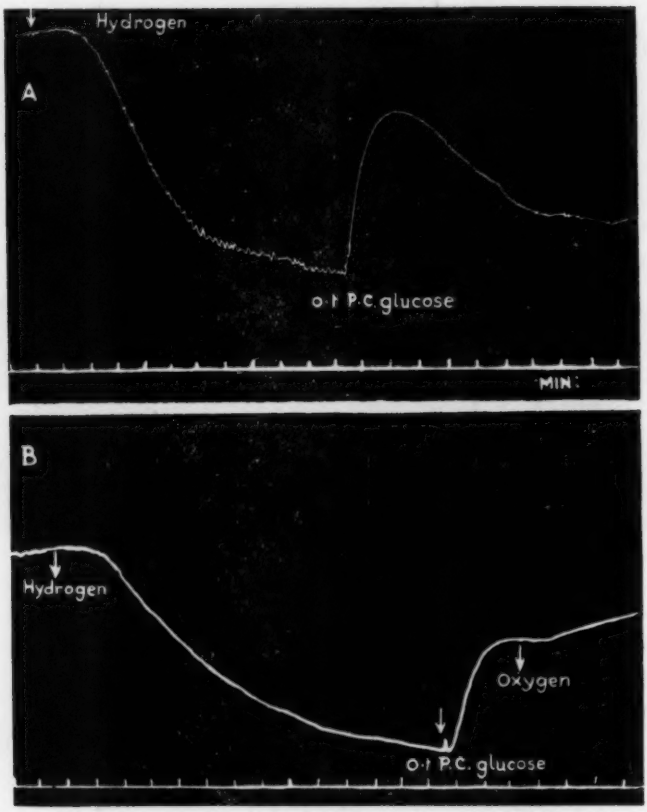

FIG. 4. *Fowl's gut.*—Effect of asphyxia, glucose and oxygen on tone.

If the asphyxia is continued for some time (beyond 10–20 minutes) then the tone again begins to increase. This is the asphyxial contraction described previously (Singh and Singh, 1947, 1948), which is rapidly abolished by oxygen in the frog's stomach, but very slowly in the dog's stomach. In the fowl's gut, this asphyxial contraction is hardly noticeable. Asphyxia thus causes two kinds of contractions; one immediately, and the other after some time. The first asphyxial contraction is usually absent. Hereafter, the second contraction will be referred to as the asphyxial contraction.

The asphyxial contraction has very interesting properties. Glucose, like oxygen, has now inhibitory action. Glucose may be added to the saline just when the tension begins to rise, or when it has risen half way fully. In the former case glucose may prevent the asphyxial contraction (Fig. 6)

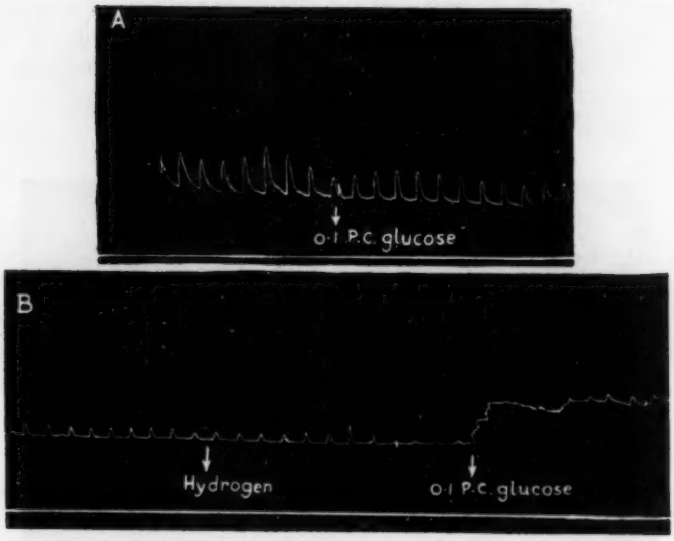

FIG. 5. *Dog's stomach.—A.* Effect of glucose in the presence of oxygen. *B.* Effect of glucose in the absence of oxygen.

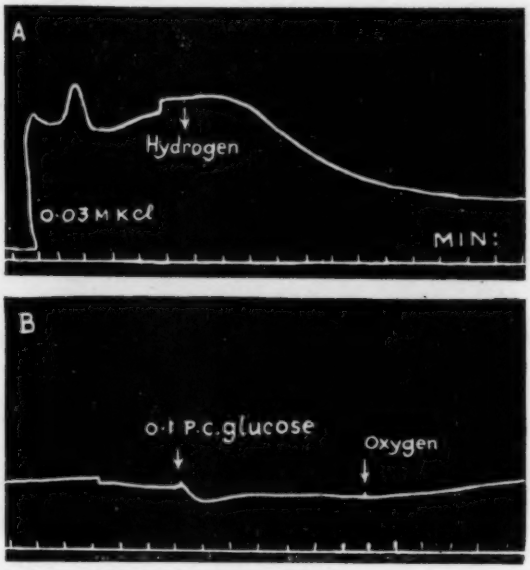

FIG. 6. *Fowl's gut.*—Effect of glucose on alactic tone. *B.* Continuation of *A.* Note contrast with Fig. 4.

and in the latter case, it diminishes the rate of rise of tension (Fig. 7) and if the tension has fully developed, it causes slight relaxation (Fig. 8). Glucose thus antagonises the asphyxial contraction but not very powerfully. This is important, otherwise the glucose normally present in the blood of an intact animal would prevent the asphyxial contraction.

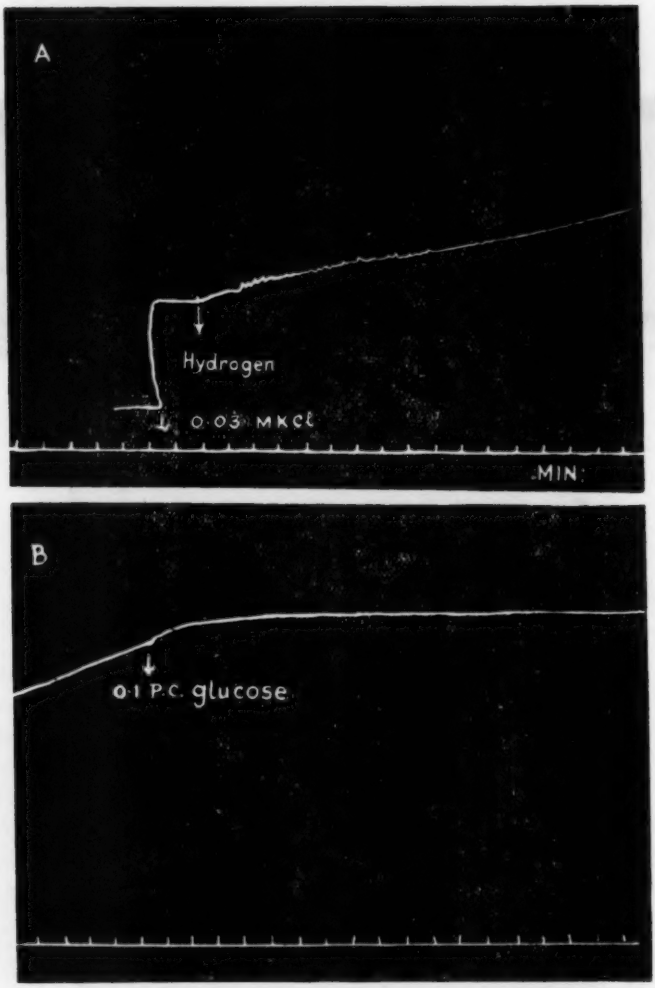

FIG. 7. *Dog's stomach.*—Effect of asphyxia and glucose on tone. *B.* Continuation of *A.* In *A*, drum stationary when potassium is added, till maximum tension results.

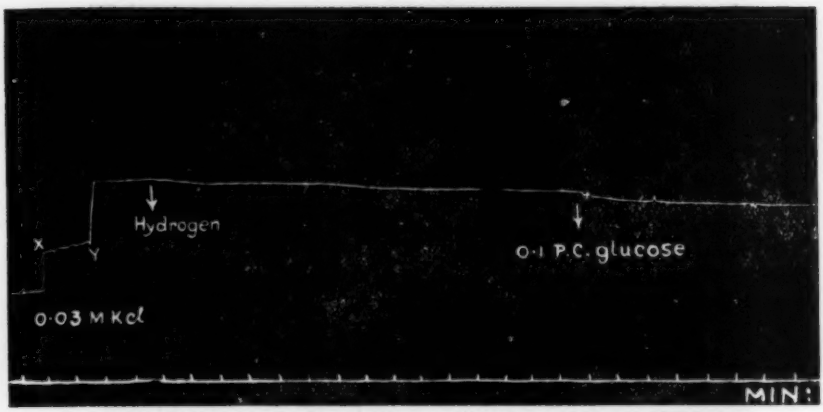

FIG. 8. *Dog's stomach.*—Effect of asphyxia and glucose on tone. Drum stationary at *X* and *Y*.

The above experiment shows that the normal state of the myosin molecule is that of shortening, but is kept extended by energy derived from the production of lactic acid. This is further shown by the action of iodoacetic acid. When the muscle is poisoned with the drug the tone in dog stomach increases. This shows that normally there is a state of tonic inhibition and upon this background further contraction is produced (Fig. 9).

Glucose changes the properties of the asphyxial contraction. In the absence of glucose oxygen inhibits the contraction, but in its presences, oxygen augments the tone. Glucose thus causes some fundamental change in the properties of the muscle. These states of the muscle may be termed as lactic and alactic asphyxial tones in the presence and absence of glucose respectively. The former tone consumes energy while the latter does not.

The change produced by glucose from the alactic to the lactic stage may be very rapid (Fig. 3) or may take about a minute or longer (Fig. 10). Thus the alactic tone may be changed into the lactic tone immediately, so that the second mechanism takes over from the first without interruption in the tension or the process may take about a minute or longer. The taking over by the second mechanism is shown by the effect of oxygen as mentioned above. The inhibitory action of glucose, thus may not be apparent, owing to the rapidity of change of state of the muscle. An interesting point is that if now the muscle is asphyxiated, it loses the tension that has been developed by the alactic mechanism.

An important property of the muscle in the asphyxial contraction is shown by the effect of potassium. If potassium had been added previously

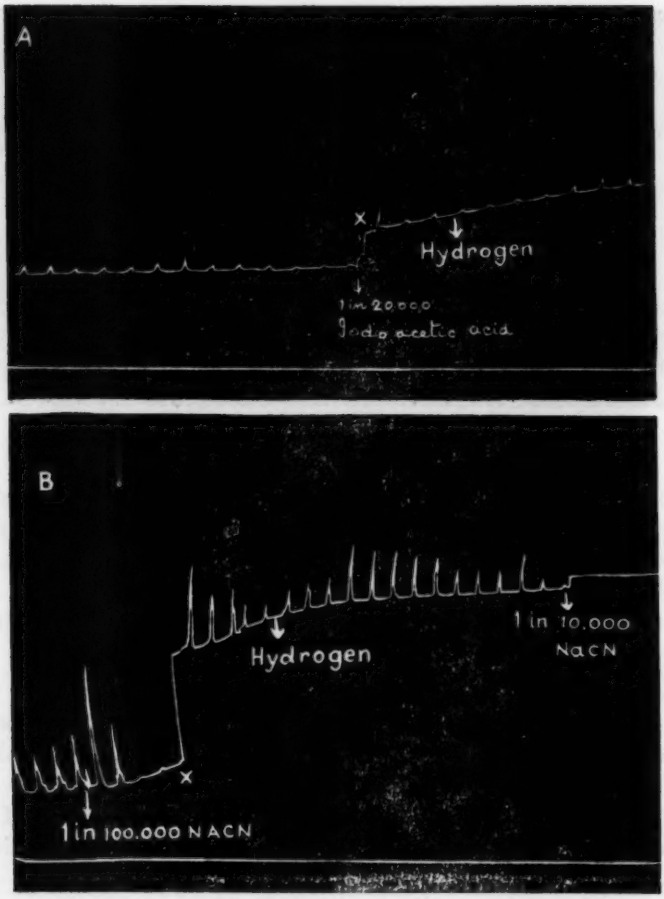

FIG. 9. *Dog's stomach.—A.* Effect of iodoacetic acid (1 in 20,000) on tone. On adding I.A.A. drum stationary for a while at *X*. *B.* Effect of 1 in 10^5 NaCN on tone, 5 minutes after adding, drum stationary for a while at *X*.

to the saline, its removal has now no effect, that is, the muscle does not relax, which it does ordinarily under ærobic conditions. This state in which the muscle does not relax, though the stimulant is withdrawn has been described previously (Gokhale and Singh, 1945; Singh 1946). The asphyxial contraction is therefore independent of the stimulant. The muscle now relaxes slowly (Fig. 11).

The slow relaxation of asphyxiated muscle is due to contraction and not the result if increase in viscosity. This is shown by the observation

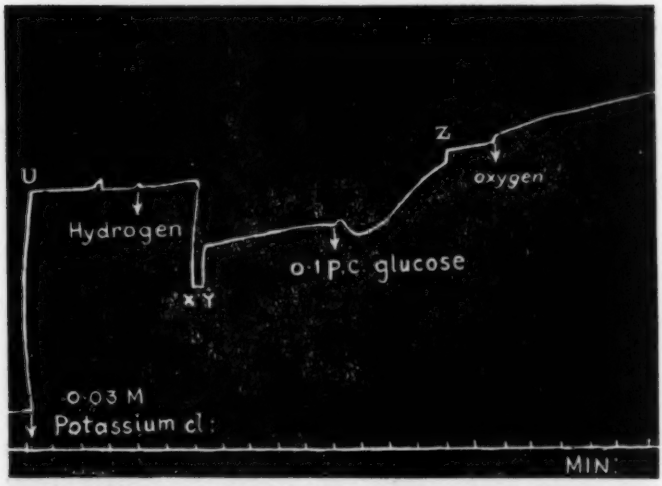

FIG. 10. *Dog's stomach.*—Effect of asphyxia on tone. Drum stationary at *U, X, Y* and *Z*.

that once the asphyxial contraction begins, withdrawal of potassium does not stop the contraction (Fig. 12).

The asphyxial contraction is hastened by prior stimulation either by potassium or by alternating current. This is due to exhaustion of the muscle by such stimulation. Sometimes on asphyxiation, the contraction begins immediately, the energy reserves presumably being very low. The asphyxial contraction is hastened by iodoacetic acid, which deprives the muscle of energy released from production of lactic acid; pH 6 has similar action.

During the asphyxial contraction the muscle is not completely exhausted of all energy reserves, as shown by the fact that the muscle responds to alternating current. It appears, therefore, that the muscle is kept relaxed by the lactic acid mechanism, at least in the dog's muscle, as shown by the specific effects of glucose. When this mechanism is exhausted, then the asphyxial contraction occurs, though energy is still available for contraction through other metabolic mechanisms. This is in agreement with the view that *rigor mortis* occurs in striated muscle when glycogen disappears (McDowall, 1946). *Rigor mortis* of striated muscle, and the asphyxial contraction of unstriated muscle, are therefore related. Normally when unstriated muscle dies, it does not show any contraction like *rigor mortis* of striated muscle, but the asphyxial contraction passes into such a state on death of the muscle.

Does the asphyxial contraction occur normally ?—The asphyxial contraction is not a terminal phenomenon, as it does not occur in a muscle dying in

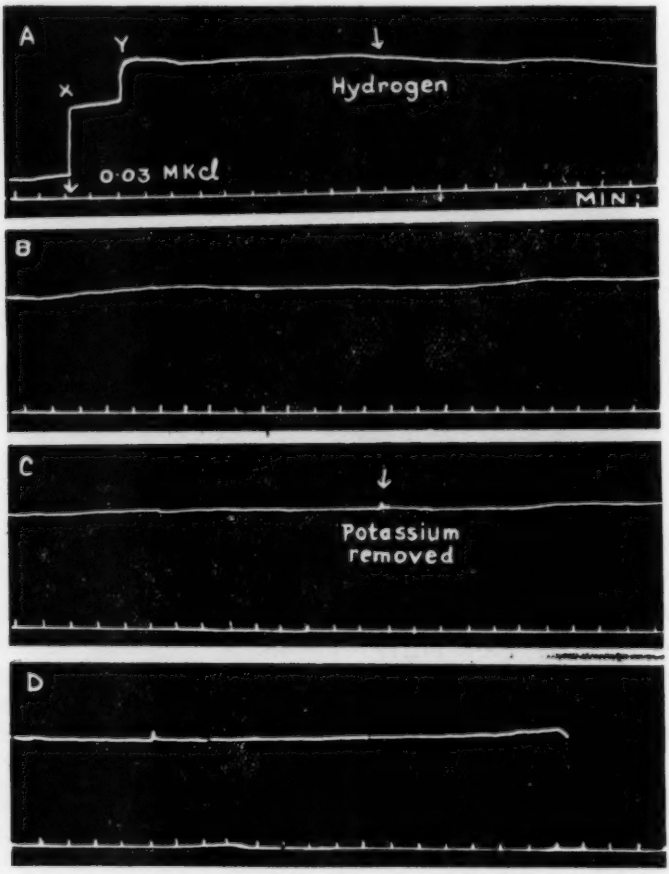

FIG. 11. *Dog's stomach.*—Effect of asphyxia on tone. Drum stationary at *X* and *Y*. *A, B, C, D* are continuous records.

saline. The chief properties of the asphyxial contraction are (1) that it does not diminish on exclusion of oxygen or addition of sodium cyanide, 1 in 10,000 (Fig. 9). (2) It is converted into ordinary tone by glucose. These two properties are shown by muscle removed fresh from an animal, so that the asphyxial contraction appears to be a normal phenomenon. It is also produced by stimulants such as potassium, barium, acetylcholine, iodide, nitrate, thiocyanate, *even in the presence of oxygen* (Figs. 7, 8, 11, 12). This is also proved by oxygen consumption experiments (Lovalt Evans, 1926; Rao and Singh, 1940). Thus the state of the muscle as found in the asphyxial contraction can be produced by the agencies other than asphyxia.

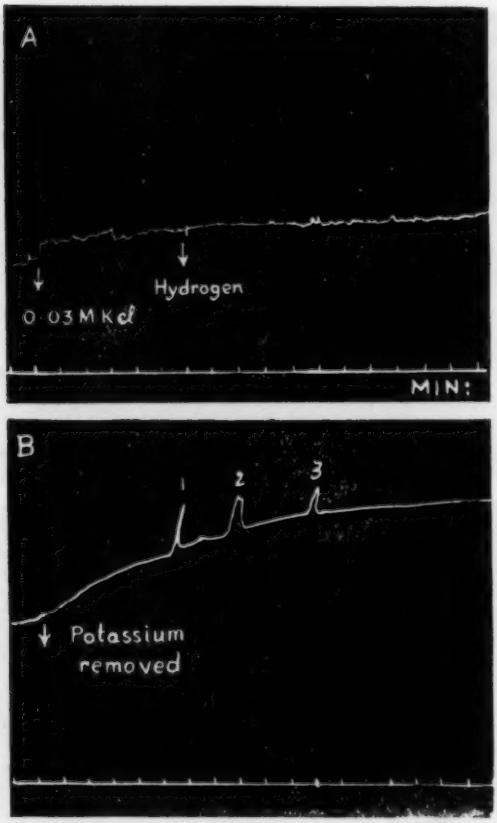

FIG. 12. *Dog's stomach.*—Effect of asphyxia or tone; stimulation by alternating current at 1, 2, 3. *B.* Continuation of *A.*

Similar mechanism, as described for unstriated muscle, appears to exist in striated muscle. Thus Langley (1913) showed that striated muscle may continue to be contracted, though the stimulant is withdrawn.

Some muscles, such as rabbit's and fowl's gut, show predominantly the non-asphyxial tone while others, such as dog's stomach, show the asphyxial one. Usually, there is a mixture. Ordinary twitch contractions and non-asphyxial tone are superposed upon the asphyxial one, the degree of the latter being variable.

Effect of cyanide.—Sodium cyanide produces effects similar to those of asphyxia 1 in 10,000 NaCN, produces immediate fall of tonus, which may be followed by partial recovery and then a further fall of tonus (Fig. 13). The

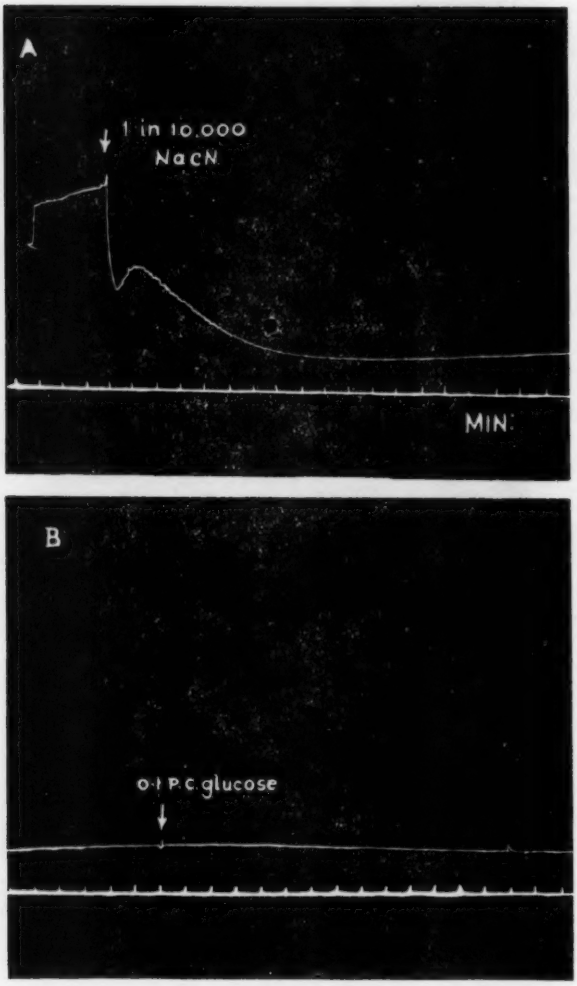

FIG. 13. *Fowl's gut.*—Effect of cyanide on tone. *B* Continuation of *A*.

partial recovery appears to be the same as the asphyxial increase in excitability. Like asphyxia, the effect of cyanide is variable. Some muscles relax greatly, while others are hardly affected. After relaxation, the muscle begins to contract. This contraction, as with asphyxia, is variable. It may contract immediately on addition of cyanide (Fig. 9) or may show only a very slight contraction (Fig. 13). As with asphyxia, glucose has a dual effect on the muscle treated with cyanide. Shortly after addition of cyanide, the

effect is stimulatory. After about 10 minutes, this effect is lost and the action may become inhibitory. Glucose then changes the alactic to lactic tone, as evidenced by the increase of tone and revival of excitability.

Small concentrations of cyanide (1 in 10^5) make the muscle more sensitive to oxygen lack, as shown by the asphyxial increase in excitability.

In the fowl's gut, 1 in 10,000 sodium cyanide causes relaxation and reduction of spontaneous contraction. These effects are further enhanced if oxygen is withheld. This shows that oxygen is also made available to the cells, by a system other than the cytochrome one (Fig. 15), or they contain a cyanide resistant cytochrome.

Inhibition

Gross and Clark (1923) observed that the asphyxiated gut did not respond to adrenaline. Prasad (1936) found that adrenaline had a powerful inhibitory effect on the activity of the gut muscle asphyxiated in presence of glucose or poisoned with iodoacetic acid. Further, Singh (1942) has shown that there are two kinds of inhibition, one produced by electric current and the other by drugs and ions; so far, the effect of asphyxia on the former inhibition has not been investigated.

Effect of asphyxia on electrical inhibition.—Electric current produces three kinds of inhibition, the first one during the flow of the current, the second on cessation of the current, and the third after the lapse of a certain interval after cessation (Singh, 1942). Any of these inhibitions can be studied. The third inhibition is due to a subnormal phase following contraction, and corresponds to indirect inhibition in the central nervous system; the first one corresponds to direct inhibition. In the guinea pig's uterus in alkaline solutions, stimulation with direct current produces beautiful inhibitory twitches; these can be produced by alternating current in the dog's stomach if the animal is killed with an overdose of ether and chloroform. In the fowl's gut, the third kind of inhibition is ordinarily produced. In the guinea pig's uterus, frequent stimulation may cause a gradual decrease of tonic contraction, just as frequent stimulation producing contraction may cause a rise in tonus.

When oxygen is excluded, the inhibitory twitches decline just as the contraction twitches and are similarly restored by glucose (Fig. 14). Glucose is ineffective in the presence of iodoacetic acid. When the muscle fatigues to electric current, introduction of adrenaline produces inhibition. Tonic contraction can also release energy not only for the twitch contraction (Singh and Singh, 1948), but also for the twitch inhibition, as shown by the

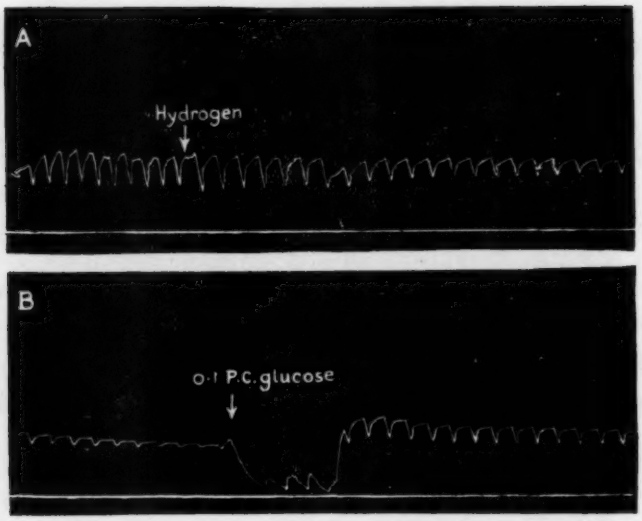

FIG. 14. *Guinea Pig's uterus.*—Inhibitory twitches by direct current 14 volts for 10 seconds per minute. *B.* Continuation of *A.*

fact that mild increase in tonus increases the inhibition (Singh, 1942). In the frog's stomach, the inhibition produced by adrenaline and ammonium decreases in the absence of oxygen and is restored by glucose. After asphyxia, on the reintroduction of oxygen, the inhibitory twitches, just as the contraction twitches, are temporarily suppressed (Fig. 15). The above results on inhibition resemble exactly those described for contraction. They are in agreement with the oxygen consumption experiments. These results suggest that the ærobic metabolic mechanisms for tone and inhibition are antagonistic.

When the muscle passes into alactic tone, then inhibition is not produced. Inhibition is produced however if the muscle is in the lactic tone; hence the beneficial effect of glucose. Thus the contradictory findings of various observers are reconciled. In the experiments of those observers in which the asphyxiated muscle did not respond, it must have passed into the second stage; in the experiments of other observers, in which the muscle responded, it must have been in the first stage.

Adaptation.—Adaptation is identical with inhibition (Singh, 1945), therefore it is affected identically as inhibition by asphyxia. It was found that cyanide was very powerful in diminishing adaptation (Singh, 1938). Asphyxia produces identical results. When the muscle is continuously

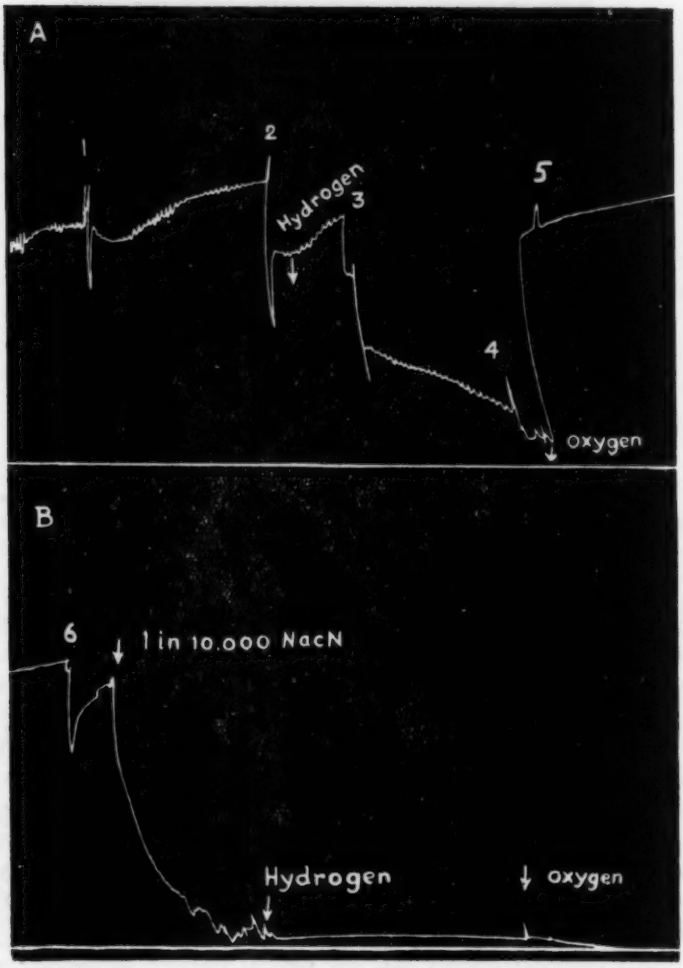

FIG. 15. *Fowl's gut.*—Inhibition following contraction by alternating current at 1, 2, 3, 4, 5, 6; note at 5 it is absent. Also effect of sodium cyanide and hydrogen. *B.* Continuation of *A*. When oxygen introduced drum stationary for 5 minutes in *A*.

stimulated with alternating current, potassium or acetylcholine, it fails to relax if in alactic tone (Fig. 16); as energy is required for the maintenance of tension, the opposite happens if in lactic tone. This is the first instance in which I have found that adaptation and fatigue are affected oppositely in unstriated muscle, that is, in the absence of oxygen.

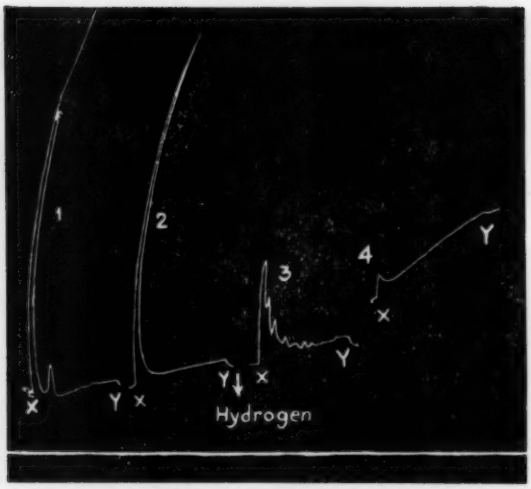

FIG. 16. *Dog's stomach.*—Continuous stimulation with alternating current from *X* to *Y* for 3 minutes. 1 and 2 normal contractions; 3 after 10 minutes of asphyxia; 4 after 20 minutes of asphyxia. Drum stationary in between contractions.

DISCUSSION

These experiments show that one of the functions of the glycogen lactic acid system is to keep the muscle relaxed. Then how does the same system cause contraction of myosin. Either there are two different kinds of contractile proteins, one of which is relaxed and the other contracted, or in the same protein, there are chemical groups which are acted upon differently by lactic acid. The other possibility is that the entire function of the energy liberation in muscle is to keep the muscle relaxed like imparting potential energy to a stretched spring. This process will have to be increased when the muscle tends to contract, hence the increased liberation of energy would not be due to the contraction *per se*, but due to increased tendency to relaxation while the muscle is contracting.

It would therefore appear that the normal state of the muscle whether in relaxation or contraction is a dynamic equilibrium between two opposing forces. This might perhaps account for the presence of a dual nerve supply to unstriated muscle. In cardiac muscle, it is known that both the sympathetic as well as the vagus are in a state of tonic activity simultaneously.

SUMMARY AND CONCLUSIONS

1. Unstriated muscle shows two kinds of tone, ærobic and anærobic.

2. The anærobic tone is further divided into two classes, one which requires energy and the other that does not; these have been termed lactic and alactic tones respectively.

3. The ærobic and anærobic mechanisms for tone and twitch are not identical.

4. Glucose has a stimulatory action on the lactic tones and inhibitory on the alactic tone.

5. Glucose converts the alactic into lactic tone.

6. Normally there is a state of tonic inhibition.

7. The asphyxial contraction is independent of the stimulant.

8. Tone similar to that produced by asphyxia, is also produced by other stimulants, so it appears that the asphyxial contraction is the mechanism by which the muscle can keep up tension without expenditure of energy normally.

9. Sodium cyanide produces results similar to those of asphyxia.

10. Sodium cyanide does not entirely abolish activity, which is further decreased by oxygen lack.

11. Inhibition is arrested by asphyxia; glucose causes restoration. On reintroduction of oxygen there is paralysis of inhibition.

12. Adaptation is diminished by asphyxia.

References

Gokhale, S. K., and Singh, I. .. *Proc. Ind. Acad. Sci.*, 1945, **21**, 202.
Gross, L. and Clark, A. J. .. *J. Physiol.*, 1923, **57**, 457.
Langley, J. N. .. *Ibid.*, 1913, **47**, 159.
Evans, C. Lovatt .. *Physiol. Rev.*, 1926, **6**, 358.
McDowall, R. J. S. .. *Hand-book of Physiology and Biochemistry*, London, 1946, 45.
Prasad, B. N. .. *J. Physiol.*, 1936, **86**, 425.
Rao, M. S., and Singh, I. .. *Ibid.*, 1940, **98**, 12.
Singh, I. .. *Ibid.*, 1938, **92**, 241.
——————— .. *Ibid.*, 1939, **96**, 367.
——————— .. *Ibid.*, 1940, **98**, 155.
——————— .. *Ind. Journal Med. Rev.*, 1942, **30**, 629.
——————— .. *Proc. Ind. Acad. Sci.*, 1944, **20**, 195.
——————— .. *Ibid.*, 1945, **22**, 123.
——————— .. *Ibid.*, 1946, **23**, 58.
——— and Singh, S. I. .. *Ibid.*, 1947 *a*, **23**, 312.
——————— .. *Curr. Sci.*, 1947 *b*, **16**, 259.
——————— .. *Proc. Ind. Acad. Sci.*, 1948, **27**, 127.
———, and Singh, S. I., Muthana, M. C. .. *Ibid.*, 1947, **25**, 51.
Watt, Dumke and Comroe .. *Amer. J. Phy.*, 1943, **138**, 610. Quoted from Best, C. H. and Taylor, N. B. "*Physiological Basis of Medical Practice*," London, 1945, 348.

A NEW METHOD FOR THE ISOLATION AND STUDY OF MICROORGANISMS FROM DETERIORATED TEXTILES

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Introduction

The isolation and study of microorganisms represent a fundamental approach to the wider investigation of the problem of microbial deterioration of various stores, particularly textiles. The methods hitherto used for the isolation of microorganisms have been based on the principle of planting the damaged materials on suitable medium.[1, 2] In view of the fact that microorganisms responsible for deterioration belong to different groups and vary widely with respect to requirement of food, pH, degree of growth, etc., isolation involves aseptical planting of numerous replicates in a variety of media. This procedure apart from being lengthy and tedious, imposes limitations of test materials. Further, it does not enable one to distinguish the causative organisms from superficial or laboratory contaminants. This method, moreover, does not indicate the relative abundance or association of various organisms in the deterioration of a material. A new technique is described in the present paper which obviates these limitations. This is based on the principle of obtaining on a microscope slide an impression of microorganisms present in the deteriorated material.

The New Method

1. *Preparation of Slides.*—Fresh test pieces are carefully washed with sterile water to remove superficial impurities; replicate strips $1\frac{1}{2}'' \times \frac{1}{2}''$ are cut with sterile scissors and planted with mildewed surface on to clean microscope slides smeared with Mayer's albumen fixative. After 24 hours, the strips are carefully peeled off, when depositions of different groups of microorganisms appear in the form of an impression print on the surface of the slides (Figs. 1 and 2).

If the test pieces are derived from exposed textiles, in which aerial hyphæ and fructifications are absent, or from old dried up material, they are first repeatedly washed with sterile water in a flask and then incubated under aseptical conditions. (Temp. 88° ± 2° F.; R.H. 96 ± 2%.) After

the revival and growth of organisms, small strips are planted on slides as in the procedure described above.

One set of slides without staining is directly mounted in Canada balsam for the examination of the relative abundance and association of organisms in the material. A second set, after fixing by flaming, is stained according to the usual Gram stain technique for the examination of bacteria. A third set is used for the isolation of microorganisms.

2. *Isolation of Microorganisms.*—Impression slides are washed gently with sterile water and placed on a sterile Petri dish. Microorganisms are then scraped off and removed to a flask by repeatedly washing the slides with sterile water. A series of dilutions is made with the suspension of organisms and plated out on Thornton's,[3] Nitrate-sucrose Agar[4] and Waksman's media[5] for the isolation of bacteria, actinomycetes and fungi respectively. Cellulose destroying organisms may be readily distinguished and isolated by plating in Omeliansky's medium[6] containing cellulose suspension.[7]

After the growths of microorganisms in plates, they are compared with those on the slides; Laboratory contaminants are thus eliminated while non-causative organisms present on fabrics may be easily determined from the paucity of their number on the slides.

Some Applications of the Impression Technique

The study of the various stages of growth and reproduction in unicellular algæ, particularly chlorophyceæ, is essential for their identification. The usual method of examination of culture is rather slow and laborious. Further, the different stages are not always readily available in a culture solution. In the application of the new technique, a piece of fabric is allowed to remain for a week in an algal culture solution. After the fabric becomes coated with algæ, it is allowed to remain suspended partly in air and partly in the culture. This induces a rapid development and maturity of algæ above the level of the solution. An impression from this region shows different stages of algæ development and reproduction.

In some fungi, the structure of sterigmata, mode of their branching, attachment of spores, etc., are important distinguishing characters. The usual method of examination by transferring the culture to a slide, results in considerable distortion, and breaking up of some of the delicate structures, leading to the loss of distinctive features. In the new technique a slide smeared with a fixative is gently pressed over a young culture in a Petri dish and incubated for a few days. The slide is then removed and may be examined for detailed vegetative and reproductive structures (Fig. 3).

A further important application of this technique lies in the preservation of a fungus culture as a Herbarium material. A great deal of the value of a culture is lost because of its evanescent character and particularly its liability to saltate on repeated subculturing. The "Petri dish culture method" of Wiltshire,[8] as also its modification,[9] would appear complicated. In the application of the new technique, impression of a culture from Petri dish is taken as described above. A permanent preparation will readily enable one to check the purity and variation of subsequent subcultures and compare the different species. A subculture may be readily obtained by inoculating a suitable medium with the scrapings from an impression slide.

Summary

The methods adopted hitherto for the isolation of microorganisms from tendered textile materials, involve considerable practical difficulties. A new technique, whereby the impression of the microorganisms is obtained on a slide, is described. The technique is simple in execution and is free from the disadvantages of the earlier methods. Some practical applications of the new technique are pointed out.

Acknowledgements

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Bibliography

1. .. "Method of Testing Biological Deterioration of Service Materials," *Office of Scientific Research and Development, Washington, Report* 6056, 1945.
2. .. "Studies of the Deterioration of Textiles under Tropical Conditions in Canal Zone," *ibid.*, 4807, 1946.
3. Thornton, H. G. .. *Ann. Appl. Biol.*, 1922, **9**, 241.
4. Waksman, S. A. .. *Soil Sci.*, 1919, **8**, 71.
5. ———— .. *J. Bact.*, 1922, **7**, 339.
6. Omeliansky, W. .. *Centr. Bakt.*, 1902, II Abt., **8**, 226.
7. Fred, E. B., and Waksman, S. A. *Laboratory Manual of Microbiology*, McGraw-Hill Book Company, Inc. N.Y., 1928, 69.
8. Wiltshire, S. P. .. *Trans. Brit. Mycol. Soc.*, 1930, **15**, 93.
9. ———— .. *Ibid.*, 1935, **19**, 259.

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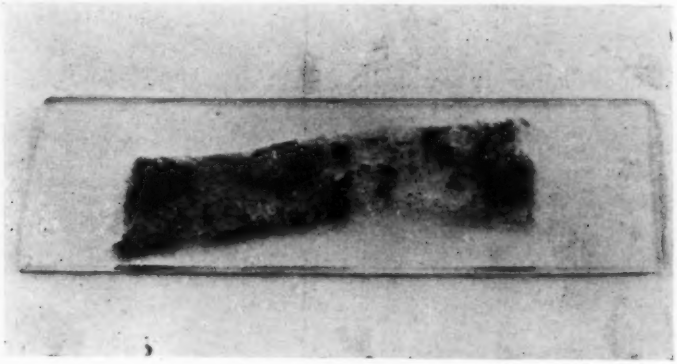

FIG. 1. (Nat. Size)

Impression from mildewed Canvas on slide

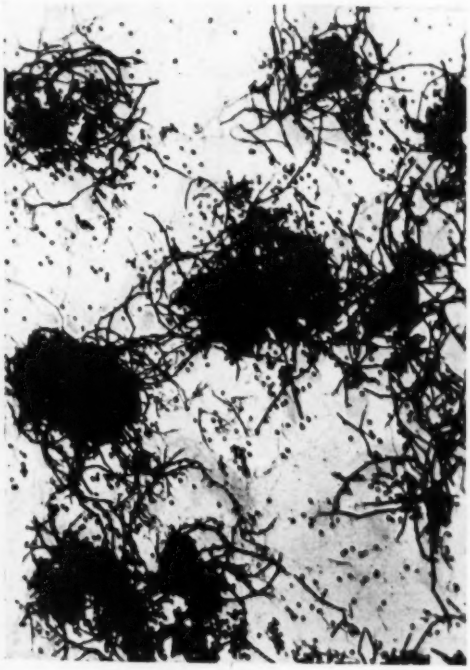

FIG. 2 (×100)

Impression of *Chaetomium globosum* from fabric

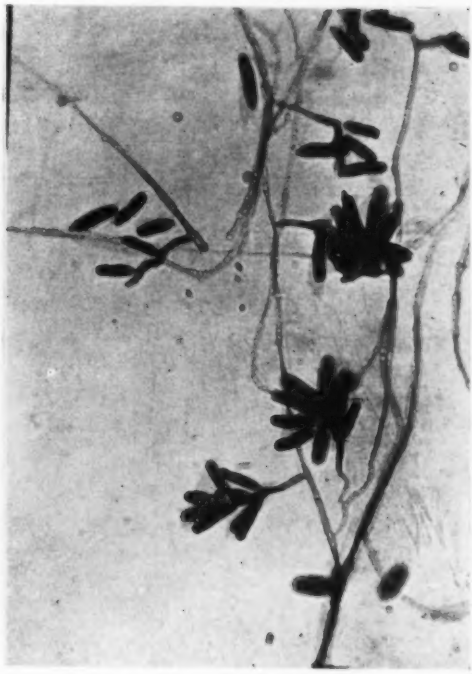

FIG. 3 (×200)

Impression of *Helminthosporium* sp. from Petri dish culture

THE EMBRYOLOGY OF *CARIDINA LAEVIS* HELLER *

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With 85 Figures and 1 Photomicrograph

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Introduction

This paper embodies the results of an investigation which was started in September 1940 in the Zoological Laboratory of the University College,

* Part of a thesis approved for the degree of Doctor of Science of the University of Madras.

Trivandrum. There has been an interruption of nearly eighteen months in the continuity of the work. This happened as a result of my transfer from the department in July 1943. However, it was fortunately made possible to resume the investigation in January 1945 when I came back to the laboratory where it was completed by April 1946. I am deeply indebted to the University of Travancore for affording me the facilities to pursue the work and for the encouragement that I have received both as a teacher and a research worker.

Caridina is a fresh-water macrurous Crustacean occurring abundantly in Trivandrum. The availability of the form was not the chief consideration which prompted me to select it for an embryological study. Though the embryology of the Decapoda, on account of their being the most important and dominant group among the Crustacea, has received a large amount of attention even from the last century, there are several points which have never been touched upon by previous workers, and several others, which, though they have been made the subject of more than one investigation, are still enveloped in no small measure of doubt. It was therefore considered worthwhile to investigate the embryology of a typical Decapod and try to elucidate the salient features thereof, with a view to relate them to the important points that have emerged from recent work on other groups of the Crustacea including the two previous studies of the author (Nair, 1939, 1941). *Caridina lævis* was found to be ideally suited for this purpose. This small shrimp which is available throughout the year and which can be reared without difficulty in the laboratory belongs to the family Atyidæ among the Caridea. It is characterised by the possession of large, heavily-yolked eggs which are carried on the pleopods of the mother till they hatch out in an advanced stage of development. A complete series of stages from the zygote to the newly hatched larva has been obtained and studied in detail. Abundant material for a study of the post-embryonic development is also available and it is hoped to give an account of this aspect of the life-history of the shrimp on a later occasion. The present paper deals exclusively with the embryonic development, *i.e.*, till the time of hatching.

ACKNOWLEDGMENTS

My best thanks are due to Rajyasevapravina Dr. K. L. Moudgill, Director of Research, University of Travancore, for the keen interest that he evinced in the work from its very beginning, and to Dr. S. M. Manton for valuable advice and suggestions. I am indebted to Dr. B. N. Chopra of the Zoological Survey of India for the identification of the shrimp and to Mr. M. A. U. Menon of the Public Health Laboratory, Trivandrum, for helping

me to take out the photomicrograph of the segmenting egg. My colleague Mr. R. Parameswara Aiyer has also been of considerable assistance to me towards the latter part of the work and I sincerely thank him.

Historical Resumé

The literature on the embryology of the Decapoda is vast and extremely confused. A number of forms have been studied in various parts of the world, the earlier attempts in this direction dating back to the middle of the last century. It is doubtless that these earlier works represented very valuable contributions at the time of their publication on account of the very imperfect state of knowledge, then prevailing on the subject of Crustacean embryology in general. However in the light of more recent work with improved techniques, their value has considerably diminished and their importance to-day is, to a large extent, mostly historical. Even apart from this there are several points on which most of these works throw insufficient light, and several others in which they are definitely defective. A good deal of disagreement on important points is discernible in the accounts given by various authors. Only those among the earlier works which have a direct bearing on the trend of the investigation here undertaken, will be referred to in the course of the present account.

The best known work on the embryology of the Decapoda, and one of the earliest, is that of Reichenbach (1886) on *Astacus*. Though this is extensively quoted in text-books of embryology, the account is extremely confusing particularly with regard to the details of the earlier phases of development. I have found it almost impossible to interpret the descriptions of Reichenbach in terms of the findings of recent workers. It looks as though *Astacus* falls into a class by itself as far as the details of the early development are concerned. Unfortunately the recent work of Zehnder (1934) on the same genus, in spite of the large number of very remarkable photomicrographs given, does not help to clear up this confusion. This work concerns itself, in the main, only with the details of the external differentiation of the embryo. The work of Bumpus (1891) on the American Lobster *Homarus americanus* and of Von Bonde (1936) on the Cape Craw-fish, *Jasus lalandi*, are also wanting in several respects. The former author has to a large extent interpreted his findings in the light of the work of Reichenbach, while the latter gives little information on those points which have been considered as significant in the present investigation. Terao (1929) has given a fairly comprehensive account of the development of the Spiny Lobster *Panulirus japonicus*. Though his description of the process of gastrulation and establishment of germ layers leaves much to be desired, the paper is valuable from the point

of view of the broader outlines of organogeny. The recent work of Krainska (1935) on the early embryology of *Eupagurus* falls into line with the investigations of Cannon, Sollaud and Manton and is of great importance.

In the Caridea, fortunately, there are a good number of very descriptive papers, though here also one cannot help despairing the great confusion that prevails. Particular reference may be made to the older investigations of Kingsley and Welden, both on *Crangon*, of Ischikawa (1885) on *Xiphocaridina*, of Faxon (1879) on *Palæmonetes* and of Gorham (1895) on *Virbius zostericola*. All of them except the last mentioned author have dealt with the entire course of embryology while some have even included in their account their observations on post-embryonic development also. The treatise of Ischikawa is of special interest to the present investigation as it deals with a form which is closely allied to *Caridina*. Gorham's paper embodies the results of a study in Crustacean cytogeny and, as such, is of limited importance as far as general embryology is concerned. It is, however, significant in that it provides yet another example, besides *Xiphocaridina*, where total cleavage has been recorded.

Among this wealth of published work on the embryology of the Caridea the most important as also the most recent is the treatise of Sollaud (1923) on the embryology of the Sub-Family Palæmoninæ. It is an elaborate paper dealing with the embryology, particularly the earlier aspects, of two forms, *Leander squilla* and *Leander serratus*. It goes into the minutest details of several aspects of the early embryology of these two forms and also attempts, and to a large extent succeeds, to clear the confusion that prevails in the accounts of earlier authors. In spite of the great care which this author has bestowed on the investigation as well as on the interpretation and exposition of his results, there are several points on which his paper throws insufficient light. His account of the origin and development of the mesoderm is very meagre and the accuracy of some of his observations becomes questionable in the light of the most recent critical work on Crustacea. The statement that the teloblasts form only the post-cephalic region of the body may be cited as an instance. It is also noteworthy that Sollaud was not aware of the existence of a pre-antennulary mesodermal somite in Crustacea. This discovery, originally due to Manton (1928), has acquired a great measure of importance on account of its obvious significance with regard to the complex problem of the composition of the Crustacean head. Since its first discovery in *Hemimysis* it has been shown to occur in such diverse forms as *Nebalia* (Manton, 1934), *Mesopodopsis* (Nair, 1939) and *Squilla* (Nair, 1941). An attempt will be made in the following pages to examine some of these important points.

Material and Methods

Caridina lævis occurs in plenty in the tanks and ponds in and around Trivandrum. It is available all through the year and that too in a berried condition. This, apparently, shows that the shrimp breeds throughout the year. It has, however, to be noted that there is a maximum of abundance, particularly of egg-bearing females and young ones, immediately after the monsoons. They cluster in the mud at the bottom of the tanks or hover around water weeds. Specimens fresh from the tanks have a dirty greenish-brown colour. They were collected with hand-nets after disturbing the mud and the water weeds. Females with mature ovaries or eggs attached to the pleopods were reared in glass jars containing tank water along with numerous male specimens. A few bits of water weeds usually sufficed for their food. The shrimp is a hardy form and can survive even if the water is not changed for weeks.

The number of eggs in one brood varies from 30 to 45. They have the same tint as the body of the mother but are darker. Eggs and embryos were removed from the living mother by placing it in a small quantity of water in a watch glass. Due to the insufficiency of the water, the animal will rest on its side and in this position as many eggs as are needed could be conveniently detached by a needle. The embryos can live in this detached condition for three or four hours and undergo development. Though the yolk is opaque and its quantity very large the embryo that develops on it is extremely transparent and this circumstance was made use of in making detailed observations on the living material.

For preparing sections the same technique as was successfully evolved for the work on *Mesopodopsis* and *Squilla* was adopted. Embryos were fixed, after noting their age, in B. G. Smith's fluid for 48 hours; the fixative being changed once after 24 hours. The material was then washed in distilled water and stored in 5% formalin in the dark. The large quantity of yolk present in the egg has been a hindrance in getting good sections. Whatever method was adopted the yolk crumbled on the microtome and the sections were spoiled. It was therefore necessary to remove as much of the yolk as possible from the embryo under a binocular microscope. The active part of the embryo when it is just forming on the surface of the egg occupies only a very small portion of the area and it was possible to remove a good quantity of the yolk from the fixed material without injuring the embryonic rudiment. When thus treated the material cut very well. But this method could not be adopted for the stages prior to the formation of the embryo on the surface, *i.e.*, the various stages of segmentation. In these every part of the egg is important and no portion could be dissected

away. The difficulty of sectioning the entire egg was all the greater here since the proportion of yolk to cellular material is very much higher in these stages. The difficulty was overcome by selecting eggs of the same stage and dividing them into two along various planes and sectioning both the halves together. When a number of eggs were treated in this manner and sectioned, it was possible to piece together accurate information about their structure. Material to be sectioned was dehydrated rapidly through alcohol up to 90% strength and then cleared in methyl salicylate. Before embedding it was passed through cedar wood oil. Paraffin of 58 degrees m.p. was used. Twenty to twenty-five minutes' impregnation was sufficient. Sections 8 microns thick, were cut.

A large number of whole mounts were made, particularly of the early stages. For these the embryonic area was dissected out free from yolk, stained in picrocarmine and mounted in strong glycerine.

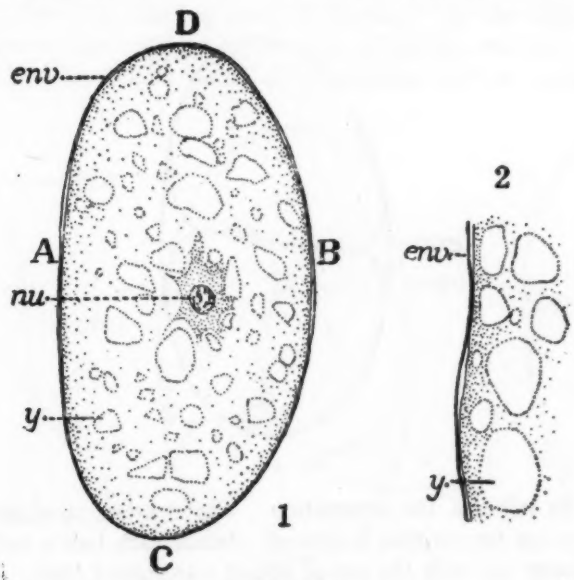
The Egg

The egg is oblong in shape and varies from greenish brown to greenish yellow in colour. It is comparatively large, measuring on the average 946 microns by 559 microns, and contains an immense quantity of yolk. In the freshly laid egg the yolk is faintly translucent and when viewed under the microscope against a strong beam of light it reveals a small central island with hazy margins. There is a thin transparent membrane closely enveloping the egg, lying so close to the surface as to be hardly distinguishable. This membrane bears thin flat ribbon-like filaments which are attached to the pleopods of the mother. Its surface is smooth. There is no doubt that this ovarian envelope is permeable as it is not necessary to remove it in order to fix the egg. Though this is generally the case it has occasionally been observed that in some broods the fixative fails to sufficiently penetrate the membrane and the eggs consequently are not properly fixed even after 48 hours' immersion. In the freshly laid condition this is the only ovarian envelope that is present. It could be seen from the very beginning, long before cleavage sets in. Even after the beginning of cleavage no other envelope could be distinguished for some time. It is, therefore, difficult to say whether it is the chorion or the vitelline membrane. However, the fact that the membrane could be clearly seen even so early as four hours before the beginning of cleavage would appear to indicate that it is the chorion.

On several occasions it has been possible to keep an entire brood of eggs under observation continuously for over four hours prior to the onset of cleavage. The changes that take place may be understood from the following description of a typical case from among these.

The new brood was observed at 10·30 A.M. The eggs completely fill their envelopes, leaving no space in between. By 1·30 P.M. all the eggs show a slight shrinkage. At either end of the long axis a narrow crescentic space appears within the ovarian envelope. Measurements show that these are brought about by the shrinkage of the egg and not by the expansion of the envelope. In some eggs a linear shrinkage of 75 microns could be noted. The interspace is filled with a clear fluid and the envelope remains distended. The eggs remain in this condition for another hour when the symptoms of the first cleavage appear.

On account of the large quantity of raw yolk that is present it has been very difficult to get good complete sections of the pre-cleavage stages. The sections crumbled in spite of every precaution that was taken. Variations of technique were also tried but without success. However, just a few entire series of sections were obtained by chance, and one of these is illustrated in Fig. 1. It shows that the egg is typically centrolecithal, possessing a large, rounded nucleus, situated at the centre in a stellate island of protoplasm continued all round as an elaborate plasmic reticulum the meshes of which are filled with yolk. The nucleus measures 45 microns in diameter. The extent of the protoplasmic island is small compared with the vast quantity of yolk and the protoplasmic network proceeding from it is thin and distinguishable only here and there in sections. A portion of the periphery



FIGS. 1 & 2

of the egg is shown under higher magnification in Fig. 2. The yolk granules extend to the very surface of the egg, there being no regular protoplasmic layer or blastema around. Only a single enveloping membrane could be seen and this remains very close to the surface.

Typically the eggs have a regular and characteristic shape. Each egg possesses a more or less straight side, a bent or curved side, a pointed end and a blunt end (Fig. 1).

Cleavage

After extrusion a few hours elapse before cleavage sets in. As extrusion generally takes place in the morning the first signs of cleavage appear after midday. The process could be conveniently studied under the microscope either by observing the brood of eggs attached to the mother, or better, by selecting a few eggs from the pleopods of the mother and placing them in clean water in a watch glass. The detached eggs continue to live and develop normally for several hours. It is delightful to watch them undergo the divisions in regular, ordered, sequence.

As soon as cleavage starts a circular shallow groove appears on the surface of the egg. It girdles the egg along its middle region in a plane at right angles to the long axis. This gradually deepens and ultimately cleaves the egg into two distinct and equal halves (Fig. 3). The whole process takes

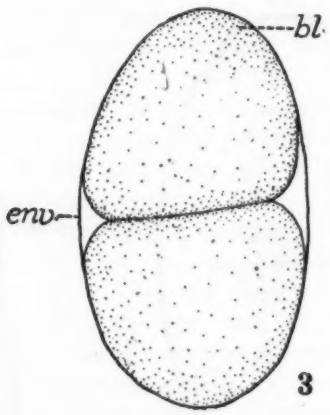
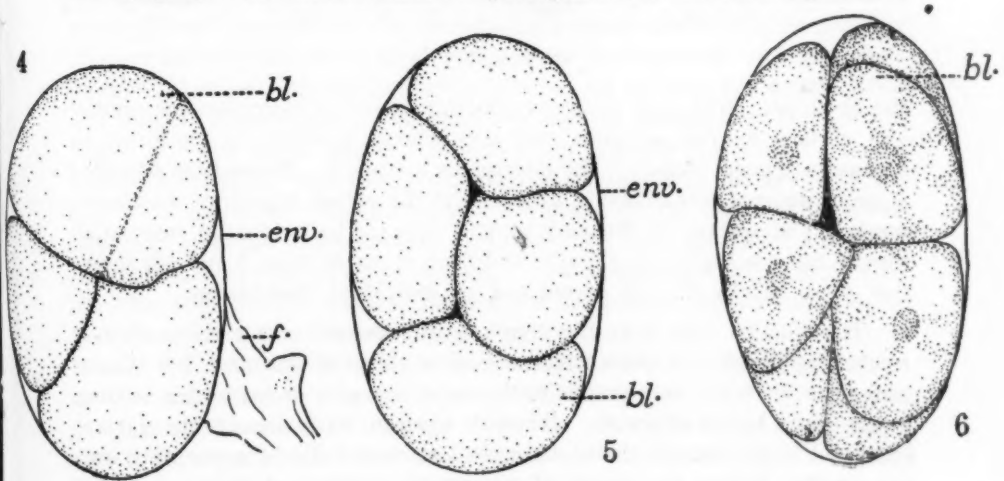


Fig. 3

from 30 to 40 minutes for completion. The ovarian envelope stretches across the cleavage furrow that is formed. Inside each half a stellate island can now be made out with the aid of strong transmitted light. This is the

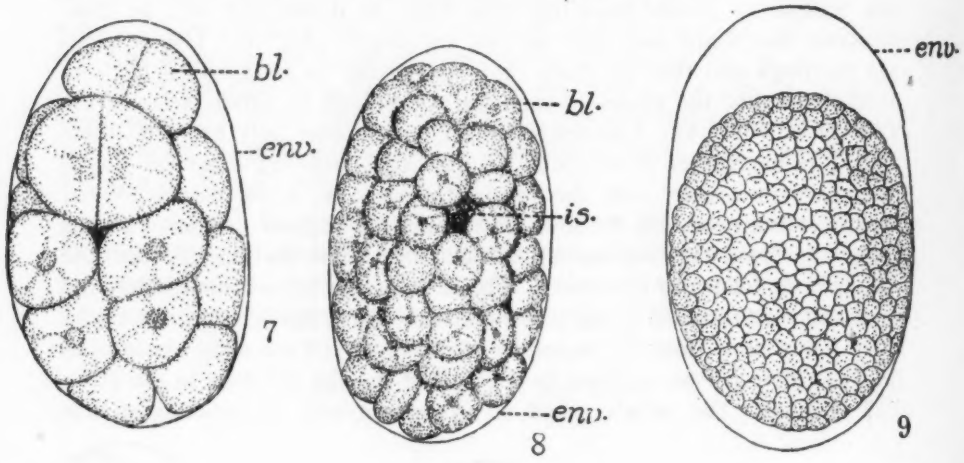
first cleavage. It affects not merely the zygote nucleus and its cytoplasmic island as is usual in the yolky eggs of the Arthropoda in general and the Crustacea in particular. The vast quantity of yolk is also affected by the division and a sharply marked furrow is visible on the surface.

The second division begins as soon as the first is completed. It takes place in an obliquely longitudinal plane. This plane is not parallel to the long axis of the egg, but is tilted at an angle of about fifteen degrees to the side. As a result of this division two blocks are cut off obliquely from each of the blastomeres that resulted from the first cleavage. In this, one side precedes the other by a few minutes. Only after the blastomere of one side has completely divided does the other begin to divide (Fig. 4). In these divisions also sharp and deep furrows are formed (Fig. 5). The divisions are thorough and their products show a tendency to get rounded off. As a result of this, the planes of cleavage are difficult to determine once the division is over. The blastomeres touch one another only here and there and leave interspaces in between. In the four-blastomere stage the blastomeres are in contact with the ovarian envelope at many points. When looked at from one side the four blastomeres may appear unequal, but this is only an illusion. They are all of equal size; those that appear small are merely being hidden by the others. The next division follows immediately and results in the formation of eight blastomeres. This stage is reached in about three hours' time from the beginning of cleavage. Of the eight blastomeres four are more or less confined to one half of the egg and four to the other half (Fig. 6). The stellate island of protoplasm with its radiating strands



FIGS. 4–6

now becomes very clear inside the blastomere. The next stage is shown in Fig. 7. Here, some out of the eight blastomeres have completed another division and the others are in the process of division. In all these divisions clear-cut cleavage furrows are formed and the resulting blastomeres appear as rounded masses (see photomicrograph). The stage represented in Fig. 8 shows about 64 blastomeres, the products of the sixth cleavage. However, after the formation of 16 blastomeres the divisions show a tendency to become irregular, so that the meaning of the term " sixth cleavage " has to be understood only in a general way. In the stage represented in Fig. 8 also interspaces could be seen amongst the blastomeres and the embryo almost fills

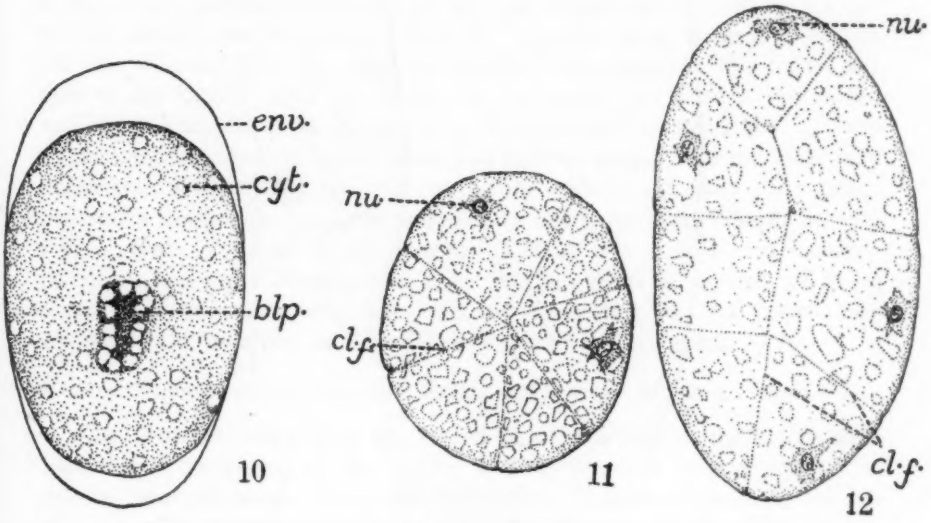


FIGS. 7–9

the envelope. With each successive division the blastomeres become smaller and, as their number increases, they press on one another and are forced to assume polygonal shapes. The interspaces disappear. Beyond the 64-celled stage it becomes very difficult to distinguish the stellate islands of the blastomeres. The surface of the embryo still appears to be cut up into small blocks, but a comparison of living and fixed material (Figs. 9 and 10) shows that these blocks do not correspond to individual blastomeres.

It would be clear from the above description that the embryo when it reaches the stage with sixteen blastomeres is a typical morula. The blastomeres can be clearly seen even with the naked eye as rounded bodies holding together as a bunch of grapes. However when the early segmenting eggs are immersed in the fixative the blastomeres lose their ball-like appearance and run together making the surface of the embryo smooth and even. But still

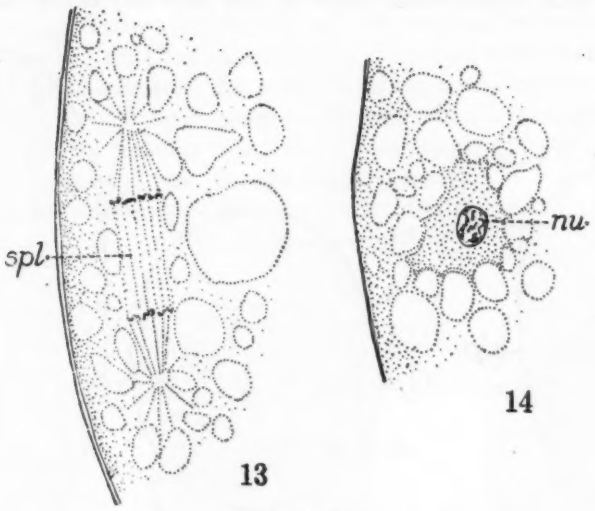
the division of the entire egg into blastomeres can be made out under the binocular as the cleavage furrows do not disappear. In section (Figs. 10 and 12) the early segmentation stages do not show any cavity within. The



FIGS. 10–12

blastomeres are prismatic in shape and they all extend to the centre. Each one is separated from its neighbours by a clear, straight boundary and appears as though it is wedged in. By introducing a sharp needle into the cleavage furrows the blastomeres in fixed material can be separated and the whole embryo taken out into its component parts. All of them are equal in size and alike in appearance. With each cleavage the stellate islands of protoplasm with the nuclei move more and more towards the surface. It has already been stated that the zygote nucleus is situated in the centre of the egg. The centrifugal displacement of the nuclei of the blastomeres is evident even from the first cleavage. In the 4-celled stage the nuclei are nearer the surface than the centre of the egg. This continues steadily so that by the time the 16-celled stage is reached the cytoplasmic islands come to lie very near the surface, some of them even touching it (Fig. 12). The zygote nucleus becomes smaller with each successive division. The spindles that are formed during cleavages are long and narrow, and lie with their axes parallel to the surface of the egg (Fig. 13). They are of the amphiastral type. The spindle that is shown in Fig. 13 belongs to the fourth cleavage and measures approximately 100 microns in length. A typical nucleus and

its cytoplasmic island belonging to the 16-celled stage is shown in Fig. 14. It measures 10 microns in diameter.



FIGS. 13 & 14

It will be noted from the above account that cleavage in *Caridina* is holoblastic, though no hollow blastula is formed. The blastomeres that are formed are all of the same size, there being no distinction into micromeres and macromeres. The large quantity of yolk is shared out equally among them. But this condition is maintained only till the sixteen-celled stage after which the divisions take place in a different manner. It was said above that planes of cleavage are at right angles to the surface of the egg. This naturally results in making the blastomeres more and more narrow as the entire quantity of yolk is shared out among the blastomeres. Beyond the 16-celled stage however this sharing out does not happen. The succeeding divisions affect only the external part of each blastomere where the nucleus is situated. Two distinct, sharply marked daughter blastomeres are formed, but only from the outer region of the mother cell. A part of the burden of yolk is got rid of in the process. The two together will have only less yolk than the predecessor had. The detached non-nucleated portions of the blastomeres accumulate in the centre of the egg. They will be bounded on the outside by a layer of small blastomeres, each enclosing some yolk and distinctly marked off from its neighbours as also from the central mass by well formed cleavage furrows. The central mass continues to show division into blocks, though these do not correspond to blastomeres as they have no nuclei in

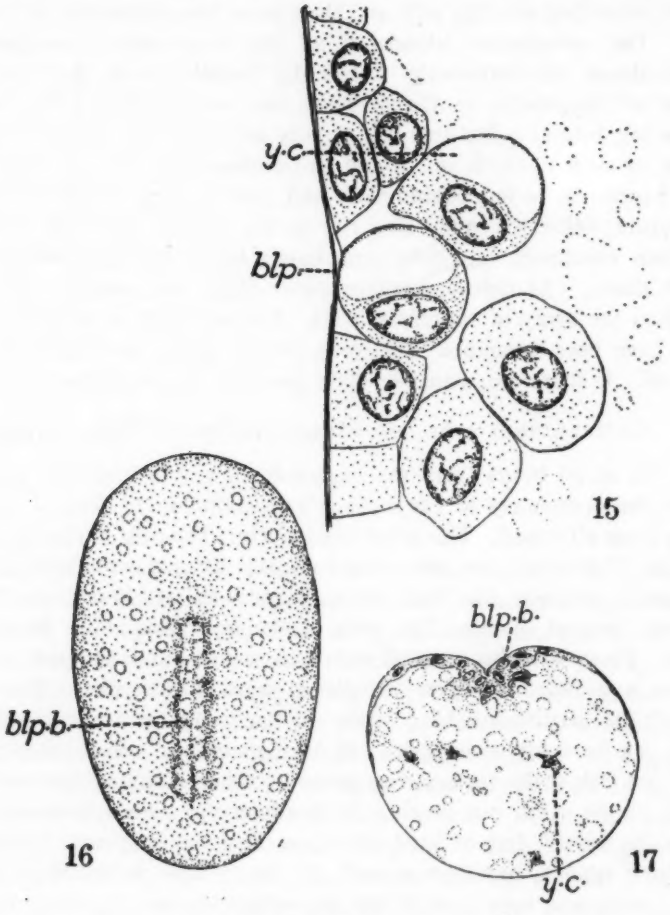
them. In this manner the blastomeres progressively get rid of their heavy load of yolk and finally, by the 128-celled stage, they arrive at the surface as sharply defined cells without any yolk. They now have no cytoplasmic strands projecting into the yolk and their inner boundaries are as even as the outer. The cytoplasmic islands of all the blastomeres condense on the surface almost simultaneously. After the 16-celled stage there is a certain amount of irregularity in the divisions and occasionally a few nuclei are seen to lag behind. But this is not very common and when it occurs the number of such nuclei is not found to be more than two or three. If these nuclei happen to be left behind early and thus lie deep within the yolk, they disintegrate; otherwise they also rise to the surface and their surrounding cytoplasm condenses into cells immediately below the layer of cells already formed there. As development proceeds these late arrivals are incorporated into the array of superficial cells. Several series of sections of these stages have been examined but only in two cases have such nuclei been observed. I, therefore, consider their presence as accidental.

Gastrulation and the Establishment of Germ Layers

In 28 to 30 hours from the beginning of cleavage all the cytoplasmic islands rise completely to the surface and condense to form a continuous cellular layer all round. This is the blastoderm. The cells of the layer are flat and thin. The central part where the nucleus is situated is thicker than the rest. It cannot be said that this blastodermal layer is formed first at one particular spot and then at others. The cells of the layer frequently show division figures. These have been noted even before they have properly condensed to form a well-defined layer completely outside the yolk. The embryos in which the blastomeres have organised themselves as a blastoderm on the surface can be easily distinguished in fixed material. When the cytoplasmic islands are below the surface, the general colour of the embryo is the same as that of the yolk, but as they gradually make their appearance on the surface the whitish tint of fixed cytoplasm becomes evident. It is first seen as isolated islands, but later spreads all over. The nucleated parts appear densely white and raised, while the rest of the surface has only a thin film of white. This film is seen to spread uniformly over the yolk and obscure it from view.

The next change that takes place is a gradual concentration of the cells of the blastoderm on one side. This comes about partly by the more rapid division of the cells of the region and partly by migration of cells from the immediate vicinity. The large-scale migration of cells over the surface of the embryo which has been observed by Sollaud (1923) in the Palæmoninæ

and on which he has laid great stress, does not take place in *Caridina*. The cells forming the general surface of the embryo show their characteristic polygonal outline throughout the course of the formation of the specialised

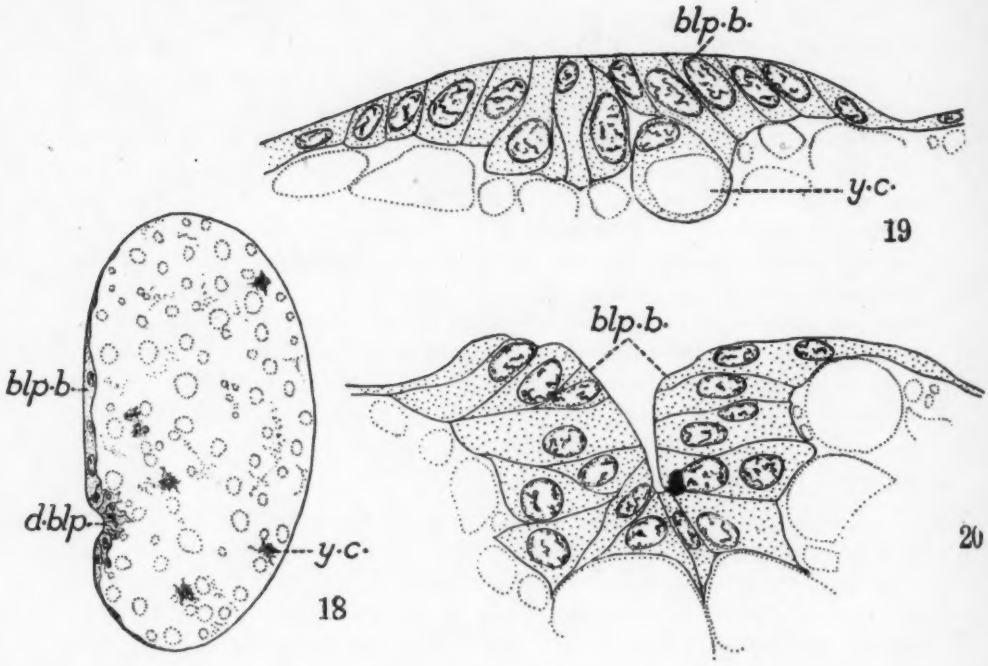


FIGS. 15–17

region referred to above. But from the immediate vicinity there is, undoubtedly, a certain amount of migration. The concentration results in making the cells smaller in extent externally. They also become thicker in sections. This region is situated on the straight side of the egg, more towards the pointed pole (Fig. 10). The thickening that is formed is at first very much restricted, consisting only of some 15 to 20 cells. It is oval in shape, thrice

as long as it is broad. After nearly 30 hours from the first cleavage the living embryo shows a series of throbbing movements at this point. The surface bubbles up over a restricted area which is slightly depressed in the middle. Obviously, this is the first symptom of the process of gastrulation. In sections this stage shows large, yolk-laden cells with conspicuous nuclei slipping down from this point into the yolk below (Fig. 15). It has also been noted that the embryo at this stage has two distinct envelopes, an outer one which has already been mentioned, and another below it, lying very close to the surface of the embryo. The latter is formed immediately after the establishment of the blastoderm over the general surface and could be clearly seen both in the living material as well as in sections. There is no evidence that this membrane was present before the establishment of the blastoderm. It has not been noted either in living material or in sections of the large number of earlier embryos that have been examined. It cannot be the vitelline membrane as it is formed only some thirty hours after fertilization. It appears to be the blastodermic cuticle of Van Benedan (quoted by Korschelt and Heider).

The cells that slip in from the thickened region of the blastoderm wander into the yolk mass. They are the yolk cells or the vitellophags. They undergo division inside the yolk and increase in number. The divisions are of the mitotic type and show a delicate achromatic figure stretching among the yolk granules. As this migration is going on the blastodermic thickening on the surface becomes more and more conspicuous. It increases greatly in length and gets narrower (Fig. 16). This narrow band of cells is nearly half as long as the whole embryo and stretches along its straight side, parallel to the long axis, with one end reaching to a point very near the pointed pole. A median longitudinal depression is formed in this band of cells, but not simultaneously along its whole length. It is first formed at that end of the blastoporal band which is away from the pointed pole of the embryo. From the sides and bottom of the depression immigration of cells continues into the yolk. The depression is at first deep and narrow, but as more and more cells composing the band sink into the yolk, it widens out and becomes shallow. The process takes place progressively towards the other end of the band, *i.e.*, in the direction of the pointed pole of the embryo. The depression that is formed at this end of the band is more conspicuous than that in the other parts (Figs. 18, 20 and 21) and remains for a longer time. The rest of the band now appears merely as a narrow thickening of the blastoderm without any depression whatsoever (Figs. 18 and 19). The depression that lingers at the end of the band measures approximately 100 microns in length and 40 microns in depth. Cells



FIGS. 18–20

continue to migrate inwards from its walls. It has been noted that the yolk cells slip in from that side of the depression which is away from the pointed pole of the embryo, while from the side nearer this pole smaller cells without yolk arise (Fig. 21). By the time this depression has elaborated itself to the full, the number of yolk cells that have wandered into the yolk mass would be large. They disperse themselves far and wide inside, the earlier ones reaching to positions near the periphery (Fig. 18). They engulf more and more yolk from the surrounding mass whose consistency changes thereby. This is particularly noticeable in the region immediately below the blastodermic band and the lingering depression at the tip of it. The yolk mass here stains less deeply than in the outlying regions. This obviously is due to the fact that yolk cells that slip in absorb their first morsels from this region and then only wander further inwards.

It will be clear from the account given that these processes relate to gastrulation and lead to the establishment of the germ layers. Gastrulation starts when the embryo is approximately in the 128-celled stage, with all

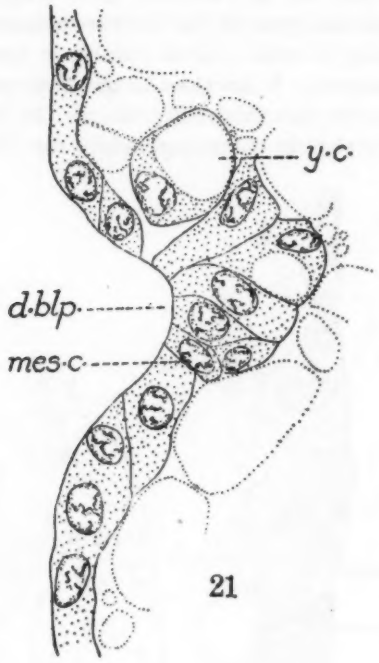
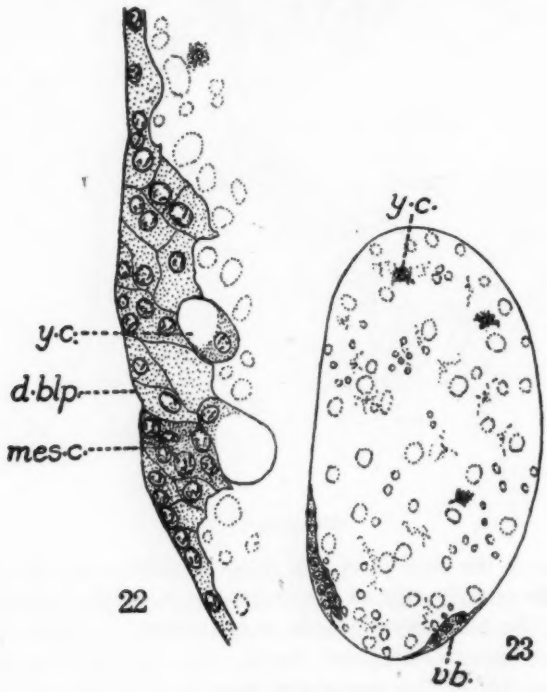


FIG. 21

its blastomeres on the surface of the yolk. It takes place by immigration of cells from the straight side of the embryo at a point which is nearer the pointed pole. The blastoporal area is at first oblong in shape but later gets modified into a narrow elongated band. The immigration of yolk cells which starts even before the blastoporal band is distinctly constituted continues along the sides and bottom of a median longitudinal depression in this band. The depression starts from that end of the band which is away from the pointed pole of the embryo and proceeds towards it, becoming more and more conspicuous in the process. It disappears rapidly except at the end of the band which is nearer the pointed pole where it enlarges and lingers for a while. This deeper lingering depression may be designated the definitive blastopore. While the immigration of yolk cells takes place from the depression all along the blastoporal band, mesoderm cells are budded off only from the definitive blastopore. They arise from that side of it which is towards the pointed pole of the embryo (Figs. 18 and 21). These cells are much smaller than the yolk cells. They do not ingest any yolk. They

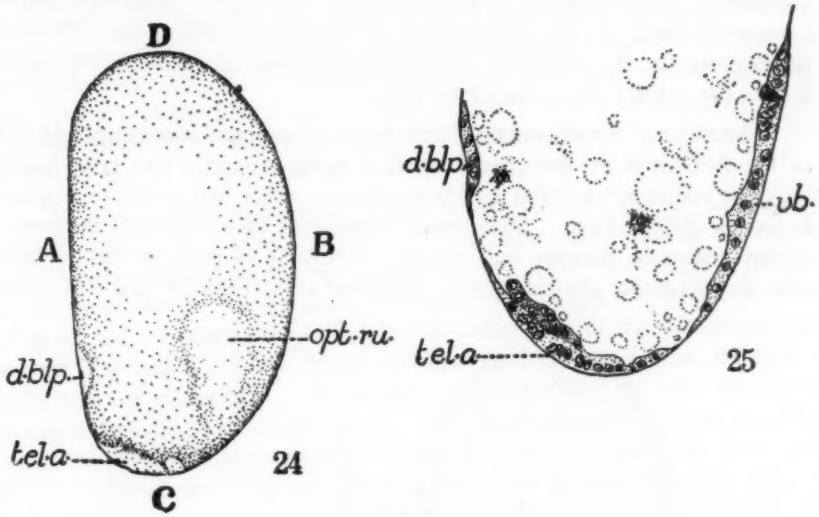
possess small nuclei and are seen to stain more deeply. They pass in and move towards the pointed pole of the embryo between the yolk mass and the superficial covering of cells. Their number is small. In Fig. 21 only one such cell is to be seen. It lies close to the blastopore while another is about to slip in from the side of the depression. In Fig. 22 which shows a sagittal section of a slightly older stage where the blastoporal depression



FIGS. 22 & 23

is fast disappearing, a larger number can be seen. The surface view of this region of the embryo at this stage does not show any organisation of the cells surrounding the blastopore. As has already been stated the cells here are more crowded together than elsewhere, but apart from this no other special features which would lead one to the inference that there is some sort of a superficial differentiation of the germ elements, have been noted. It is only from the walls of the blastopore that differentiation of these elements takes place. The differentiation of the yolk cells which ultimately give rise to the endodermal layer commences very much earlier than that of the masodermal cells. They continue to be budded off right through the

entire process of gastrulation, while the differentiation of the mesodermal cells is delayed till the last phase of gastrulation when the definitive blastopore is established. The first formed mesoderm cells pass in individually, spreading slightly as they do so. The last batch of cells, however, remains attached together and move in the direction of the pointed pole in a body (Figs. 23 and 25).

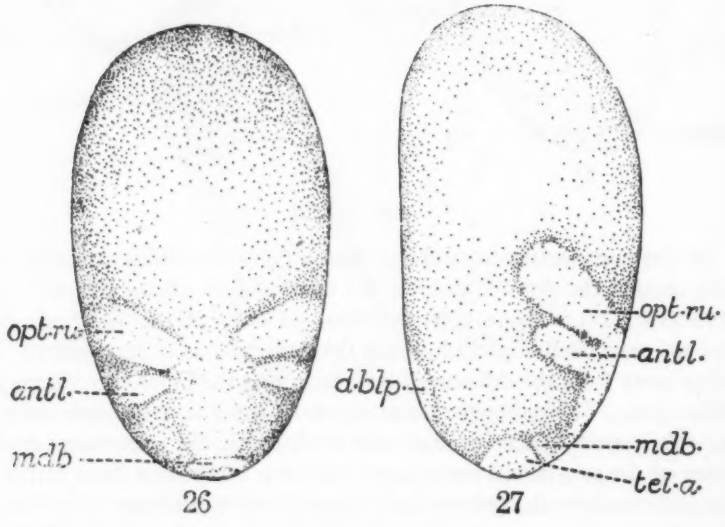


FIGS. 24 & 25

As these processes are taking place, the ectodermal covering of cells in and around the pointed pole of the embryo gets organised into a definite pattern (Fig. 24). Three concentrations of cells of the layer are formed, one at the pole itself slightly towards the straight side of the embryo, a short distance away from the definitive blastopore, and the other two, symmetrically on either side. These three concentrations are not interconnected at the beginning; only sparsely placed ground cells are found in the intervening area. The margins of the two lateral concentrations reach to about a third of the length of the embryo from the pointed pole beyond which there are only the ground cells. All the three concentrations are circular in outline and only one layer thick to start with. The mesodermal cells that are budded off from the blastopore pass towards the pointed pole of the embryo and occupy positions immediately below the ectodermal concentration that is forming there (Fig. 25). It is now possible to get an idea of the orientation of the future animal with reference to the shape of the egg. The two lateral ectodermal concentrations represent the beginnings of the optic rudiments, while the

median concentration at the pole is the region where the teloblasts get differentiated. The curved side of the embryo near the pointed pole represents the ventral side of the future animal. It is noteworthy that the optic rudiments at this stage are situated wide apart, far removed from the mid-ventral line; also that the median concentration of cells, which I designate as the teloblastic area, is formed at some distance away from the definitive blastopore. With further development the blastopore closes up and the blastoporal band on the straight side of the embryo disappears. However, the position of the definitive blastopore continues to be distinctly marked out on the surface till a much later date.

The further development of the three ectodermal concentrations leads to the formation of the characteristic V-shaped band. The cells between the optic rudiments and the teloblastic area on either side gradually aggregate to form a distinct band. Immediately behind the optic rudiment the position of the antennule becomes marked out. Similarly in front of the teloblastic area the mandible also is defined on either side (Figs. 26 and 27). There



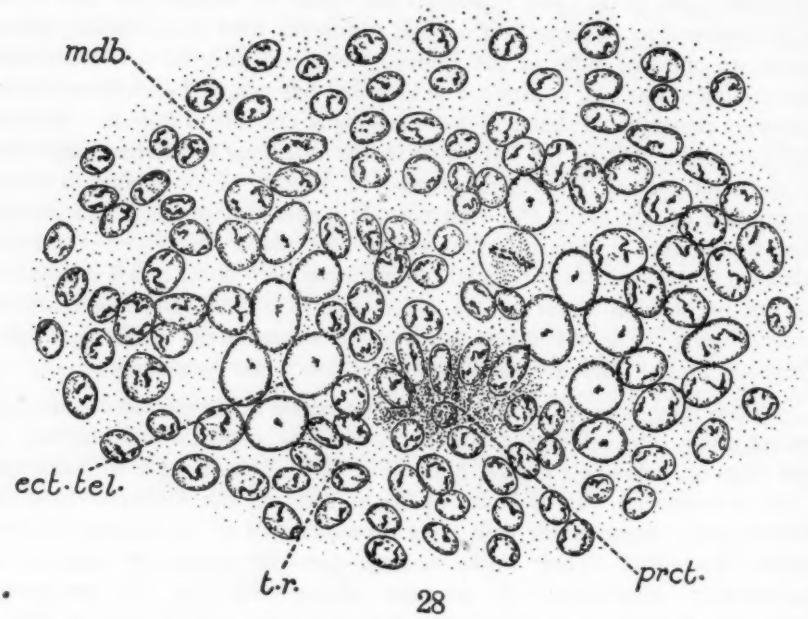
FIGS 26 & 27

is a wide interval between the antennule and the mandible which remains undifferentiated at the time these two appendages are first appearing. The arms of the V are well formed at this point also but the appendage that is to form there becomes marked out only later. The two arms of the V are not straight. The distance between them is greatest in the middle (Fig. 26).

The inner edges of the optic rudiments are nearer the median line than those of the antennules. Originally the optic rudiments were much further apart, almost on opposite sides of the embryo; but now they have come closer. Each rudiment has also contracted in area and has assumed an oblong shape. It may be remembered that they were originally circular in outline. Sollaud (1923) observes that in the Palæmoninæ the antennule is differentiated from the posterior part of the original circular thickening. I am unable to either corroborate or contradict this statement as I have not obtained the critical stage where such a differentiation might take place. However, a certain reduction in the area of the original rudiment has been noted and it is possible that this is brought about by the differentiation of the antennule from its posterior part. The mandibles are formed immediately in front of the teloblastic area. They are small and lie close to the median line.

By this time marked changes become noticeable in the median ectodermal concentration (the teloblastic area). A shallow depression is formed a short distance behind its central point (Fig. 26). This is the first indication of the proctodæum. It becomes visible as soon as the V-shaped band is differentiated. Some of the cells lying on either side of the depression also become specialised. Their nuclei enlarge and the chromatin assumes a characteristic disposition. It becomes concentrated at the periphery leaving only a small bit at the centre. Six such cells could be seen on either side of the proctodæal depression in Fig. 28. It is obvious that these cells are the ectodermal teloblasts. They arise in two groups from the sides of the proctodæum and are not found immediately in front of or behind the proctodæum. The nuclei of these cells measure 20 microns in diameter on an average, whereas those of the unspecialised cells average only 8 microns. The area that these cells enclose and in which the proctodæal depression is situated is destined to become the telson of the adult. Even when the teloblasts are in two distinct groups on either side of the proctodæum they begin to divide (Fig. 28). The longitudinal axis of the division spindle so formed is found to lie parallel to the arm of the V-shaped band of the respective side. The divisions are of the mitotic type.

Several series of sections of the embryo at this stage have been obtained. On account of the peculiar orientation of the V-shaped band around one pole of the egg, different planes of section had to be chosen for getting correct results. A section passing transversely through the optic rudiments and the antennule will not also cut the teloblastic area transversely, but only tangentially. The facts mentioned below have been fully verified by examining all these sections.

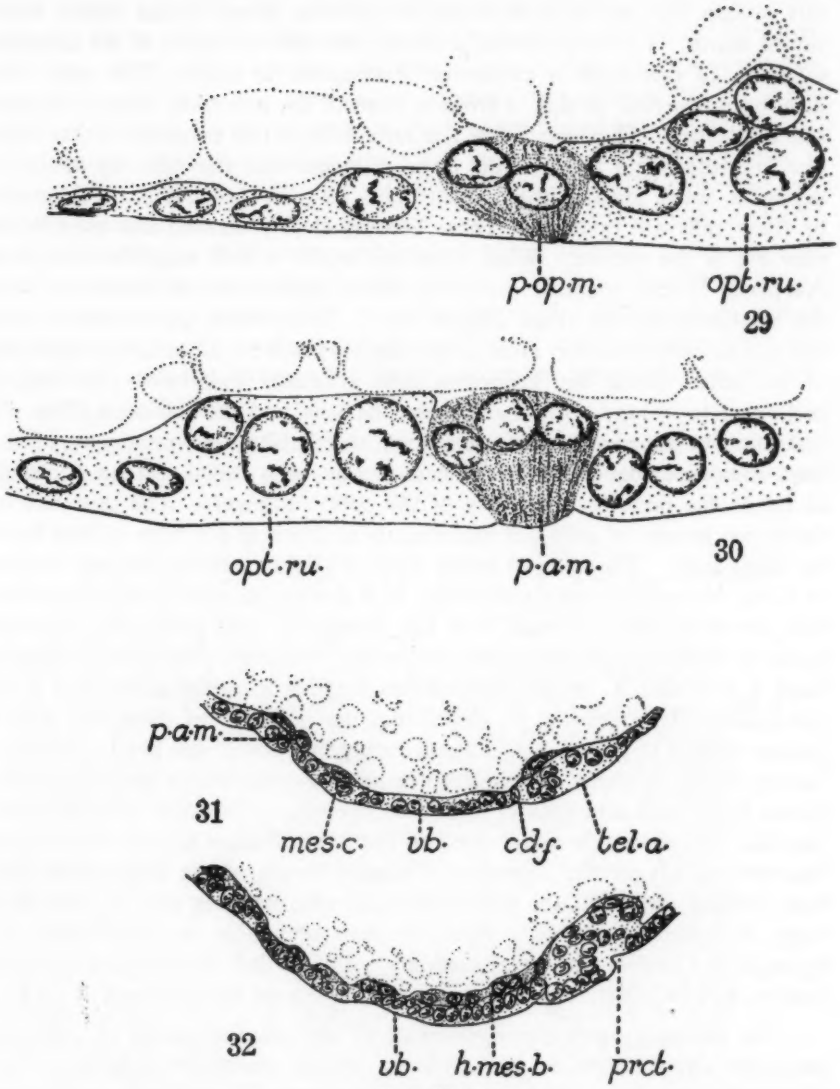


28

FIG. 28

The ectodermal layer is only one cell thick along most of the embryonic area except in the vinicity of the proctodæum where it is thicker. The mesodermal cells have passed beyond the anterior boundary of the teloblastic area and have spread forwards below the arms of the V-shaped band (Figs. 31 and 32) between the ectoderm and the yolk. The cells appear dark and thin with rounded nuclei. They are slowly aggregating together to form a regular layer below each arm of the V. These are the head mesoderm bands which supply the nauplier segments. The bands have not yet broken up into the somites of the respective antennulary, antennal and mandibular segments, but remain continuous (Fig. 32). They are confined to the region lying immediately below the inner edge of each arm of the V. As has already been stated, the ectoderm of these segments is very extensive in the transverse direction; but the mesodermal bands do not extend sideways. In the parasagittal section shown in Fig. 31 the mesoderm is hardly visible, except in the region of the antennule in front and the proctodæum behind. In the latter region the mesodermal teloblasts are yet to be organised.

A very interesting feature that has been noticed is the insinking of cells which later take up positions corresponding to and contiguous with the head



FIGS. 29–32

mesoderm bands, from the region of the V-shaped band lying in front of the antennulary segment. It may here be recalled that Sollaud (1923) has recorded the insinking of cells from various points along the V-shaped band. In spite of very careful examination of a large number of serial sections

this process has not been observed in *Caridina*, except in the region mentioned above. I have no doubt in stating that the mesoderm of the naupliar segments of this form is exclusively blastoporal in origin. The cells that have been observed to sink in from in front of the antennule have a different fate altogether. They slip below the surface from two points on either side; one immediately in front of the optic rudiment and the other immediately behind it, more towards the median line. In each spot not more than two or three cells are involved. These become clearly marked out even when they are at the surface. When examined under a high magnification their cytoplasm reveals granular striations which mark them off distinctly from the neighbouring cells (Figs. 29 and 30). Their nuclei appear smaller and are seen to shift from their more or less central positions to the lower boundary of the cells. These two groups of cells gradually sink below the surface and come to occupy positions between the yolk and the ectoderm (Figs. 25 and 31). In appearance these cells look exactly like the components of the head mesoderm bands, which as has already been stated, extend forwards as far as the posterior boundary of the optic rudiments. The posterior of these two groups of cells lies immediately in front of the edge of this band on either side. The anterior group which is slightly smaller, lies still further in front, beyond the optic rudiments, at a greater distance from the median line, on either side. Though it is not contiguous with either the posterior group or the head mesoderm band, its position with reference to the V-shaped band is such that it can be considered as forming a regular series with it on either side. There can be no doubt that the posterior of these two paired groups of cells (lying as it does immediately in front of the head mesoderm bands) is the rudiment of the preantennulary somite which has lately been shown to occur in a number of Crustacea (*Hemimysis*, *Nebalia*, *Mesopodopsis*, *Squilla*). However it is a fact that this important feature has not so far been observed in any of the numerous Decapod forms whose embryology has been worked out. But the indication is so unmistakable even at this early stage of development that I claim to have been able to demonstrate its presence in *Caridina* with great certainty. The further development of these somites will be described in detail in the course of this account.

The homology and the significance of the anterior group of cells are not quite clear. They do not give rise to any particular structures, but degenerate after some time. I call them the preoptic mesoderm rudiments for convenience of description.

The Nauplius Stage

In about 48 hours from the onset of the first cleavage the embryo develops into the nauplius stage. Externally a certain amount of contraction

in the antero-posterior axis of the embryo is noticeable. The teloblastic area with the mandibular segment immediately in front of it shifts forwards and comes to lie more towards the curved side of the egg. Similarly the optic rudiments with the antennulary segment immediately behind them slightly move back. The two arms of the V-shaped band approximate themselves more towards the median line. The wide gap in each arm of the V between the antennule and the mandible becomes considerably reduced and the antenna gets differentiated there. Between the two arms of the V, at a point in level with the anterior boundary of the antennæ, a small depression, the stomodæum, makes its appearance. The labral rudiment lies in front of it, but is not very clear yet. It consists of a thickening of cells in the median line. The optic rudiments and three naupliar appendages are distinctly marked out, but none of these has become appreciably raised from the general surface.

Posteriorly, in the teloblastic area, the number of cells has considerably increased by this time (Fig. 33). The anterior margin of the area is well

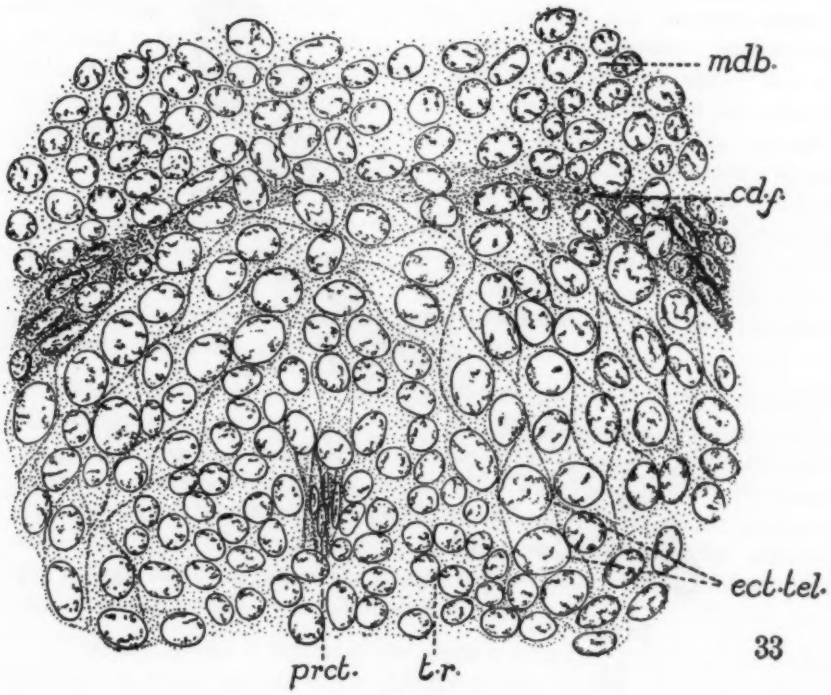


FIG. 33

marked out now by a slight semicircular depression which is deeper at the sides. This depression is very narrow and lies immediately behind the mandibular segment. This is the first indication of the caudal furrow. The teloblasts have arranged themselves in an arched row lying parallel to the caudal furrow that is forming. Three or four rows of cells with very large nuclei could be seen in this position. Their nuclei do not show the characteristic arrangement of the chromatin observed in the preceding stage; but there can be no doubt that the innnermost of these concentric rows of large cells contains the teloblasts, the outer rows being composed of their descendants. The teloblastic row is incomplete behind the proctodæal opening. The ends of the row come round to a short distance behind the

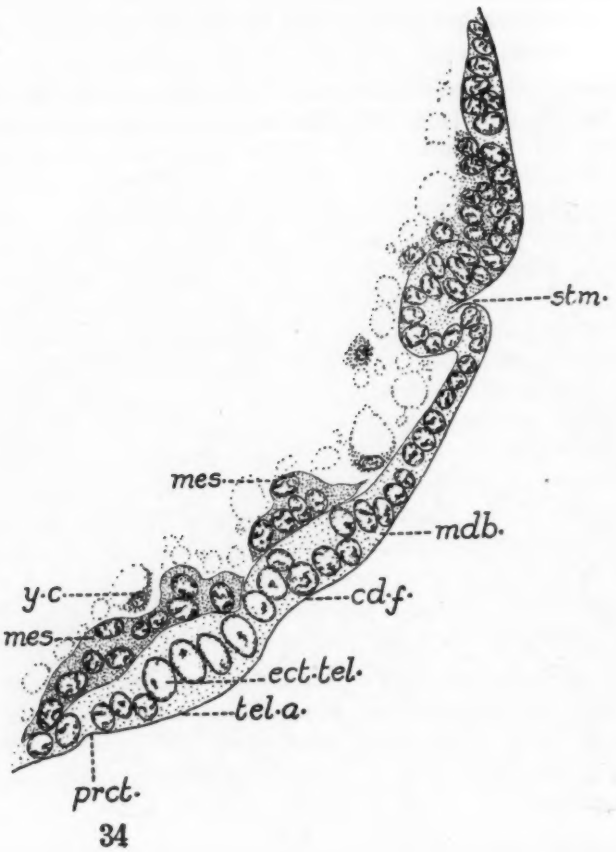


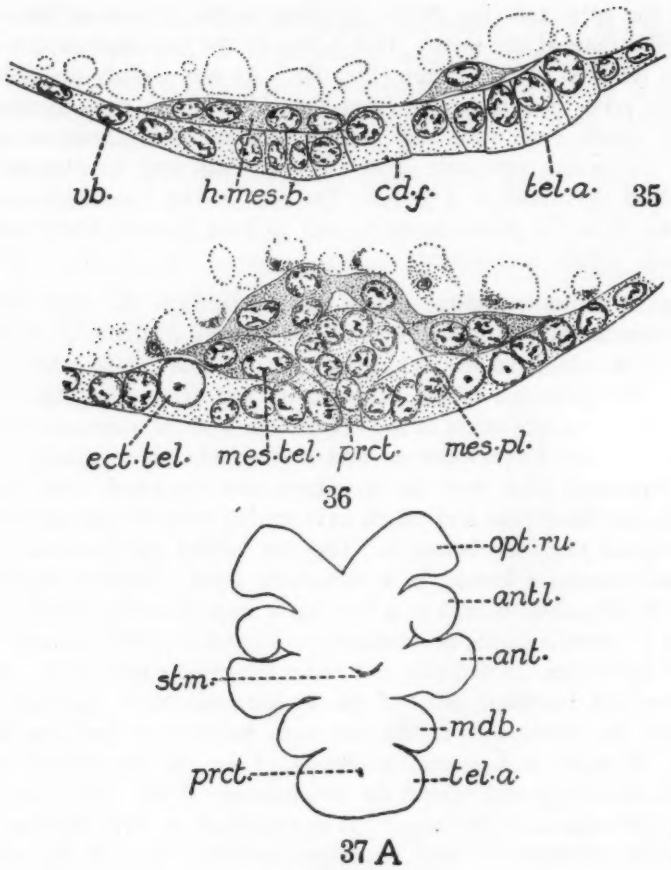
FIG. 34

level of the latter and stop there. In front, in the midventral line, the cells are smaller than at the sides. This is due to the fact that there was originally no teloblast in this region (Fig. 28). As was already pointed out the teloblasts arise in two groups from the sides of the proctodæum. They begin to divide even when they remain separate and get approximated to form a continuous row only lafer. This process may be observed in the preparation illustrated in Fig. 33. The row is just becoming continuous. In surface view the proctodæum is seen to have become restricted in area and more deeply invaginated.

Fig. 34 shows a sagittal section passing through the entire embryonic area at this stage. It is slightly oblique, passing medially in front in the region of the stomodæum, and slightly to one side in the teloblastic area behind. The positions of the proctodæum and the caudal furrow can be recognized. The mesoderm of the mandibular and the antennulary segments is clear; so also the ectodermal and the mesodermal teloblasts. The last named structures arise from the mesoderm elements which were budded off last from the blastopore and which have moved towards the teloblastic area and occupied positions below it. They lie behind the posterior edges of the head mesoderm bands, in a connected layer. Division figures could be seen in this mass of cells at a very early stage showing thereby that it is growing in extent. As the proctodæum is formed it pushes its way into this mass of cells (Figs. 36 and 38), and raises the middle part of it. As this is happening the marginal cells of the mesodermal mass gradually enlarge and form the teloblasts. It has not been possible to find out the exact number of these mesodermal teloblasts. Like the ectodermal teloblasts they also are not found behind the proctodæum at this stage, but are confined to the sides and the front. It is important to note that the row of mesodermal teloblasts is very much smaller than that of the ectodermal teloblasts which are formed very much earlier.

Fig. 35 shows a parasagittal section of the mandibular segment and the teloblastic area of an early nauplius stage. The mesoderm of the mandibular segment is seen as a regular band of cells. The ectoderm of the segment is becoming thicker. The caudal furrow is indicated as a shallow depression. The ectodermal teloblast and a few of its descendants are clearly seen in the teloblastic area. The mesodermal teloblasts have not become organised, but a single cell belonging to the margin of the mesodermal mass lying below the ectoderm in this region is visible.

Fig. 37 *A* and *B* represent the surface views of a late nauplius stage, the first in outline and the other with the cellular details. The three naupliar



FIGS. 35–37 A

segments are clearly formed and their appendages are just becoming raised from the general surface. The stomodæum appears as a crescentic slit owing to the backward shifting of the labrum. The lateral boundary of the teloblastic area does not extend beyond the edge of the mandible. The row of ectodermal teloblasts is still rather irregular, though it can be seen spreading around the proctodæum and the telson rudiment so as to meet behind. Two sagittal sections of a stage very near the one sketched in surface view are shown in Figs. 38 and 39. Only the mandibular segment and the teloblastic area are shown. Fig. 38 is almost median sagittal. The ectodermal teloblasts are seen undergoing division immediately behind the mandibular segment, whose mesodermal somite can be clearly seen in the figure. Fig. 39

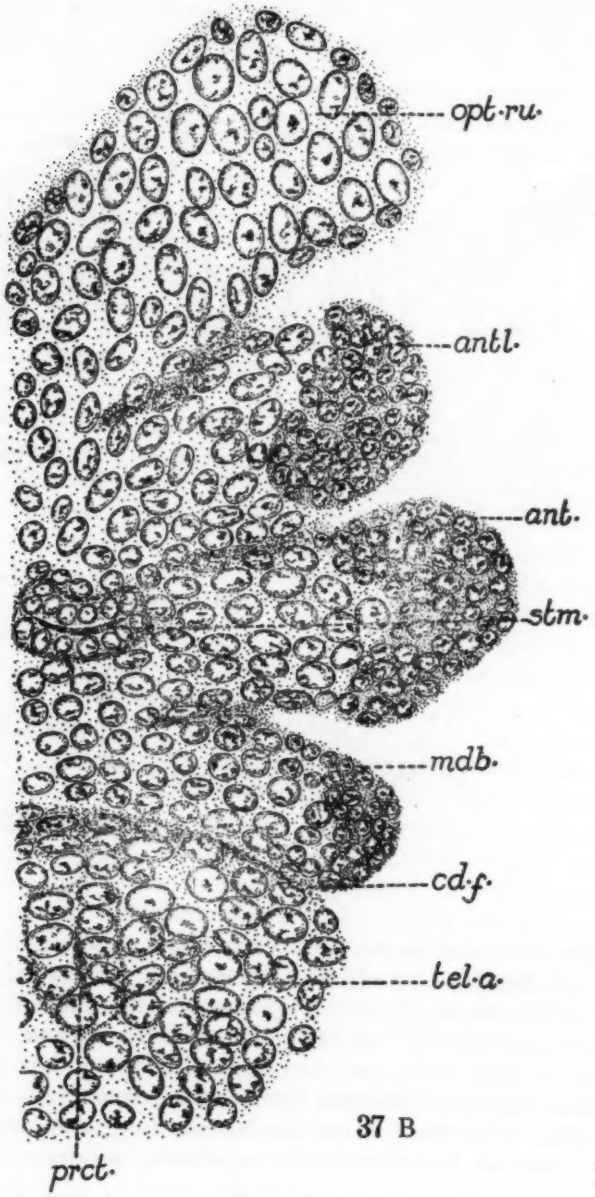
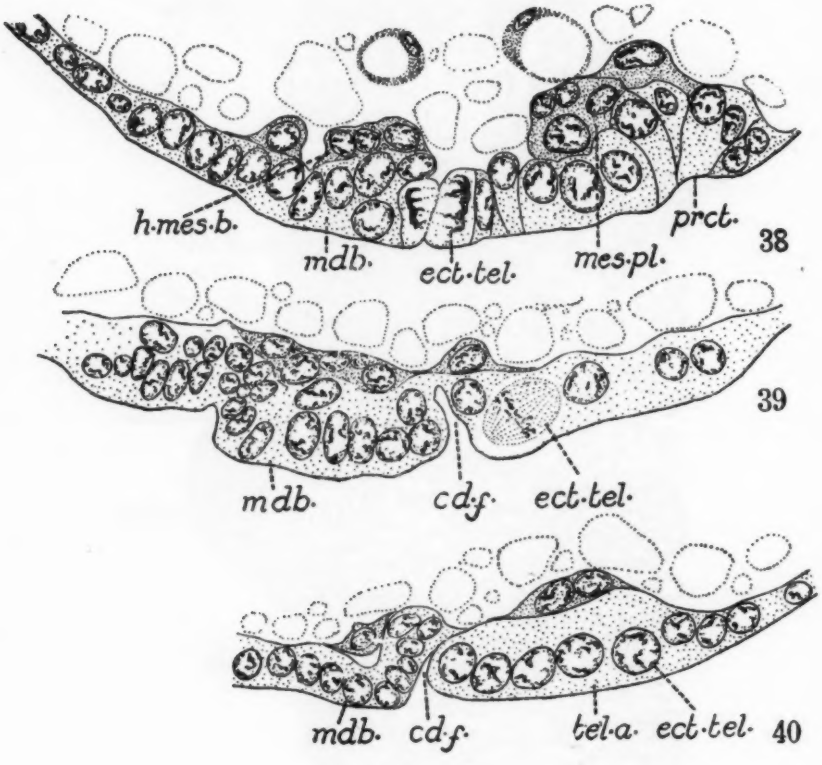


FIG. 37 B

is of a parasagittal section. It shows the mandible which is just becoming raised from the surface and also the lateral fringe of the teloblastic area.



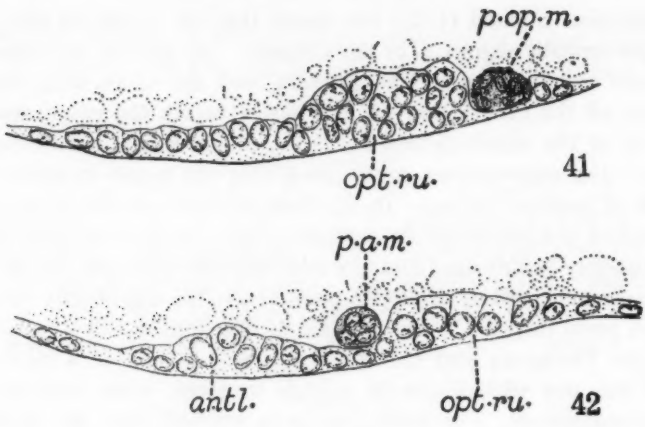
FIGS. 38–40

Here also the division of the ectodermal teloblast is in progress. The caudal furrow is well marked. Fig. 40 also represents a parasagittal section but it is of an earlier stage. Nevertheless, here also the ectodermal teloblasts are seen lying immediately behind the mandibular segment. It is likely that of the five large nuclei seen, two or three lying near the mandible do not belong to the actual teloblasts but to their descendants. In any case, it is clear that the teloblasts start their activity from a point immediately behind the posterior boundary of the mandibular segment.

These observations on the growth of the posterior region of the nauplius stage are of considerable significance. In his account of the embryology of

the Palæmoninæ Sollaud (1923) has stated that the teloblasts give rise only to the post-cephalic segments of the Caridea. He divides the tissues of the animal into two categories, the primary and the secondary, the former comprising all the tissues that are laid down before the commencement of the activity of the teloblasts and the latter, all those that are formed by the teloblasts. His observations go to show that the entire cephalic region is composed of primary tissues. In the large amount of critical work on the embryology of the Malacostraca published since the time of Sollaud's paper, it has invariably been found that the teloblasts actually give rise to the post-naupliar segments. Manton (1934) pointed out the desirability of reinvestigating this point in order to verify whether this represented a real difference between the Decapoda and the other groups. It may here be mentioned that this was one of the specific objects in mind, while undertaking the present investigation. The point has been verified with the greatest care and I have no doubt that the observation made by Sollaud does not hold good for *Caridina*. A very large number of critical stages have been examined, both in whole mounts and in sections, but no evidence as to the existence of the last two cephalic segments before the commencement of the activity of the teloblasts has been obtained. Sollaud contends that these two segments are formed in the bend of the caudal papilla from cells that lie in front of the row of teloblasts. It has been stated above that the division of the teloblasts (at least the ectodermal ones) begins even before any indication of the caudal furrow becomes visible and that even after the furrow has been marked out there is some delay in the differentiation of these segments and their appendages (Figs. 28, 37, 38 and 39). I am inclined to think that Sollaud's observation may not be correct. One important reason that prompts me to think so is the fact that he recognized the teloblasts (ectodermal) only after they had formed a regular, continuous row, stretching from side to side, whereas they actually get differentiated and even undergo divisions long before arranging themselves in this manner, *i.e.*, when they occur as two widely separated groups on either side of the proctodæum. Since the mandible has already been differentiated the teloblastic descendants that result from these early divisions, must necessarily belong to the succeeding segments. Indeed they are the elements which give rise to the two maxillary segments. It is possible that Sollaud, while studying stages where the teloblasts have arranged themselves in a continuous row, has taken these cells, found on either side, as belonging to the category of primary embryonic elements, overlooking the fact that they actually are the first descendants of the teloblasts themselves.

The condition of the two isolated groups of mesoderm cells in the neighbourhood of the optic rudiment is shown in Figs. 41 and 42. They have



FIGS. 41 & 42

become rounded masses and lie close below the ectodermal layer which has becomc continuous over them. The optic rudiment itself has become thicker and the cells in its central region show large rounded nuclei. The nuclei of the mesoderm masses, on the other hand, are smaller and darker. Their cytoplasm also stains more deeply. The preoptic mesoderm mass already shows the first signs of disintegration.

THE POST-NAUPLIAR STAGES

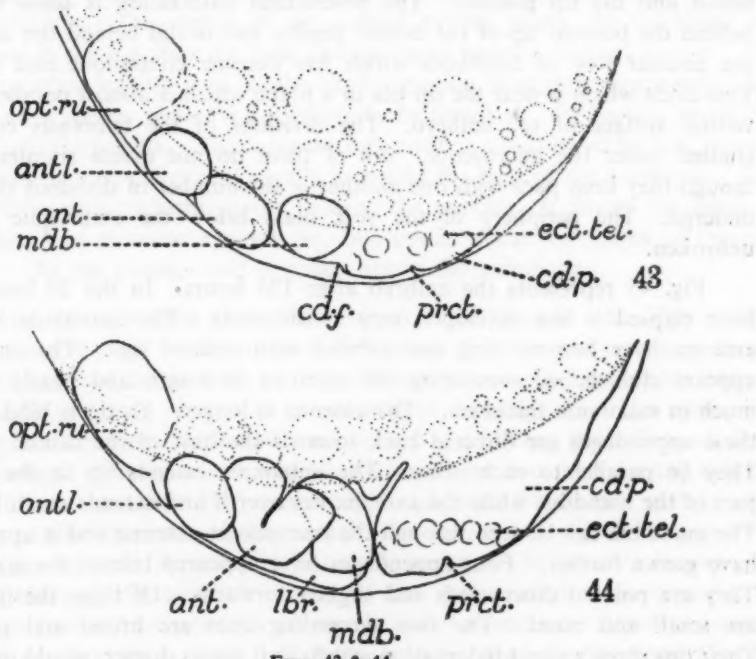
(*a*) *Observations on the Living Embryo*

Beyond the late nauplius stage the embryo becomes clearly marked out on the surface of the yolk and it is possible to follow many of the details of the further development by periodical examination of living material. In a brood the embryos at any time will all be more or less in the same stage of development. They could be detached from the mother, one or two at a time, and examined under the microscope. Several broods were kept under observation in this manner from the commencement of cleavage to the time of hatching and even beyond it so that every fact noted could be verified fully. In three cases the larvæ that hatched out in the laboratory lived for over six months in a healthy condition and underwent further development. The embryo is quite transparent and when placed in a cavity slide it permitted examination even under high power. Counting from the commencement of cleavage it takes seventeen days for the embryo to hatch out. This has invariably been the case in all the broods studied.

From this long series, seven stages have been arbitrarily selected and are shown in Figs. 43 to 49. All the details noted in the living embryo are

not shown in the diagrams as it would make them very complicated. In the earlier stages the embryonic area with its neighbouring regions alone is represented. All the embryos except the last one are sketched from the side.

Fig. 43 represents a stage which is approximately sixty hours from the beginning of cleavage. The three naupliar appendages have become raised from the surface and appear as bud-like prominences. Their tips are blunt and the inside hollow. The optic rudiments lie flat on the surface of the yolk and their outline can be distinguished only with difficulty. The labrum is visible as a rounded prominence the tip of which comes level with the base of the antenna. It overhangs the stomodæum. The antennule slightly overlaps the front edge of the antenna. The mandible on the other hand is separated from the latter by a clear gap. The caudal papilla is just becoming raised from the surface. The furrow in front of it is still not deep, but its position is clearly marked. When the surface of the caudal papilla is examined under high power the row of ectodermal teloblasts can be made out. Each teloblast is nearly twenty-five microns in size. The proctodæum appears as a well defined indentation. It is not exactly at the centre but slightly towards the back. The row of teloblasts curves round it. There is



FIGS. 43 & 44

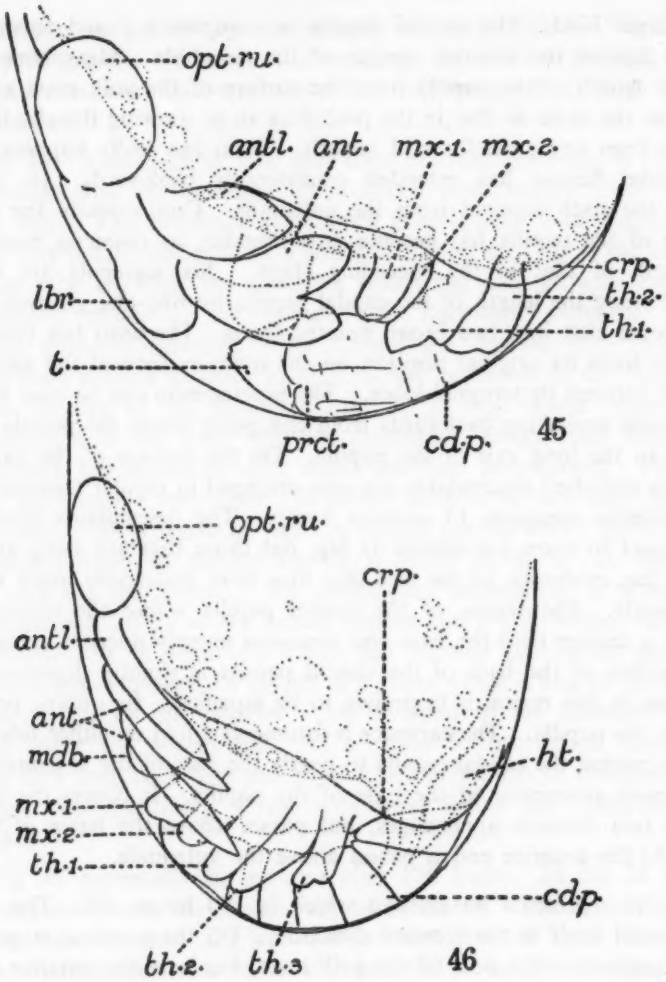
no indication as yet of the post-mandibular appendages. The boundary of the yolk mass is even and it is very near the surface showing that the embryonic tissues are still very thin. The cells are generally large and regularly arranged.

Fig. 44. This stage is over four days old (the exact age is 102 hours). The antenna and the mandible have grown considerably. The former is already bifid. This condition is obtained after the tip of the appendage has become very much swollen. As a result each lobe is as big as the base. The posterior lobe overlaps the mandible which has also grown considerably. The antenna grows in the upward and backward direction while the mandible grows downwards. The labrum has become very massive. It extends from a short distance in front of the antennule to the level of the posterior border of the mandible. Its tip is very much rounded. The stomodæum now lies level with the mandible. Behind the mandible the first maxilla has appeared as a small bud-like prominence. The caudal furrow has become very deep. It points backwards and lies behind the first maxilla. As a result of this the caudal papilla which has raised itelf prominently from the general surface is flexed forwards. Its base is very broad and the tip pointed. The proctodæal indentation is some distance behind the pointed tip of the caudal papilla and is still behind the centre of the circular row of teloblasts which has become continuous and regular. This circle which is near the tip lies in a plane which is almost parallel to the ventral surface of the embryo. The divisions of the teloblasts could be studied under the microscope. All of them do not divide simultaneously though they keep pace with one another in the number of divisions that they undergo. The periphery of the yolk mass below the embryonic area is unbroken.

Fig. 45 represents the embryo after 126 hours. In the 24 hours that have elapsed it has developed very considerably. The antennule and the antenna have become long and tubular with pointed tips. The antennule appears club-shaped measuring 100 microns in length and nearly half as much in maximum thickness. The antenna is longer. Its tip is bifid. Both these appendages are directed back towards the base of the caudal papilla. They lie parallel to each other. The antennule extends up to the middle part of the mandible while the antenna lies over it and extends much further. The mandible can be seen through the transparent antenna and it appears to have grown further. Four appendages have appeared behind the mandible. They are pointed downwards and slightly forwards. Of these the first two are small and blunt. The two succeeding ones are broad and pointed. Their tips show a slight indentation which, as it grows deeper, would make the

appendages bifid. The caudal papilla is completely flexed forwards. Its tip lies against the anterior margin of the mandible. Measurements show that the length of the papilla from the surface of the yolk mass at its base is almost the same as that in the preceding stage showing thereby that there has not been any great forward growth. What has really happened is that the caudal furrow has extended considerably backwards. Its apex lies against the sixth segment from the antennule. Consequently the proximal portion of the papilla has become more slender, its diameter being hardly one-fourth of that in the preceding stage. Six segments are distinctly marked along the length of the caudal papilla by ring-like grooves. Its tip has become cleft into two broad pointed lobes. The anus has been shifted forwards from its original position on the outer surface of the papilla into the cleft between its terminal lobes. The proctodæum can be seen as a solid rod of cells projecting backwards from this point inside the papilla. It lies parallel to the long axis of the papilla. On the surface of the papilla the teloblasts and their descendants are seen arranged in regular transverse rows. Each teloblast measures 15 microns across. The descendants lying immediately next to them are almost as big, but those that are away are much smaller due, evidently, to the fact that they have undergone many divisions subsequently. The region of the caudal papilla where the teloblasts are situated is thicker than the base and measures seventy microns across. The outer surface of the base of the caudal papilla is slightly depressed. The yolk mass in this region is beginning to be liquefied. Its outline projects a little into the papilla. The carapace is formed as a fold on either side extending from behind the caudal papilla to nearly the base of the antennule. The fold is more prominent at the base of the papilla. It covers the bases of the first two thoracic appendages, but passes above the bases of those in front. At the anterior end it passes above the antennule.

Fig. 46 represents an embryo which is 150 hours old. The embryo has extended itself in the forward direction. Till the previous stage it was almost confined to the pole of the yolk mass, but here the anterior edge of the optic rudiment reaches to the middle of its long side. The optic rudiments have become thick and massive and are folding themselves off from the surface. The antennule and the antenna have become long and tubular. The former measures 180 microns in length and 30 microns across. The lobes of the latter are of the same thickness, but are slightly longer. The mandible has not appreciably increased in size. The distance between the mandible and the anterior border of the optic rudiment is considerably greater than in the previous stage. It is here that the maximum increase in length has taken place. The gap between the mandible and the first



FIGS. 45 & 46

maxilla noted in the preceding stage has disappeared. The two appendages almost touch each other. The second maxilla and the first three thoracic appendages follow in regular succession without any gap in between. The tip of the first maxilla is slightly bifid. The first two thoracic appendages have become distinctly biramous but only one lobe of each is visible from the side. The tip of the third appendage is just becoming bifid. Behind these the rudiments of three more appendages have been formed. These

are not shown in the figure. They are small and are overlapped by the preceding appendages. Counting from the antennule seventeen segments can be clearly distinguished on the body of the embryo.

The tissues of the embryo have become thick and the yolk is diminishing in quantity in its neighbourhood. In the other parts of the yolk mass there is no change whatsoever. At the opposite pole the yolk appears absolutely inert. At the base of the caudal papilla the midgut is in communication with the yolk mass by means of a funnel-like opening. Small quantities of liquefied yolk are seen passing into the lumen of the midgut. The tip of the caudal papilla lies level with the first maxilla. It is rounded and shows a few short spines. All the appendages show slight movement at this stage. The caudal papilla jerks periodically. With each jerk the yolk at the base of the papilla is disturbed and a small quantity of it is drawn into the lumen of the gut. The heart is visible on the outer side of the gut at this point.

Fig. 47. This stage is nine days old. The tissues of the embryo have grown in thickness and the yolk has decreased in quantity. The latter shows widely separated radial striations in the region away from the embryo. An ectodermal fold has grown in between the body surface and the optic rudiments which have become larger and rounded and shows the first trace of pigmentation. Their anterior boundary extends slightly beyond the middle of the long axis of the egg on one side. The labrum shows three indentations on its surface. The antennule is long and tapers to a fine point. It shows a constriction a short distance in front of the tip which divides it into a long, thick basal portion and a short thin terminal part. The appearance of the antenna has totally changed. Its two lobes which in the previous stage were long and tube-like have become dissimilar. The upper lobe is very much longer than the lower and shows a number of annulations. It also bears a number of setæ along the lower margin. These two lobes are borne on the tip of an unjointed basal stalk. Behind the antenna six appendages can be made out externally. The mandible and the two maxillæ are rather stumpy in appearance. Of these three cephalic appendages the first maxilla alone is bifid. The three thoracic appendages lying behind these are longer and all of them distinctly bifid. The lobes are finger-shaped and are directed downwards and forwards. Each of these appendages, from the point of attachment to the body to the tip of the lobes, measures 160 microns. The caudal papilla reaches forwards to the level of the labrum. The telson is bifid. Its lobes are rounded and each bears a row of spines along the margin. When examined in a suitable position the proctodæum and the midgut could be made out through the body wall. The former is short. Though they are in contact there is as yet no communication between

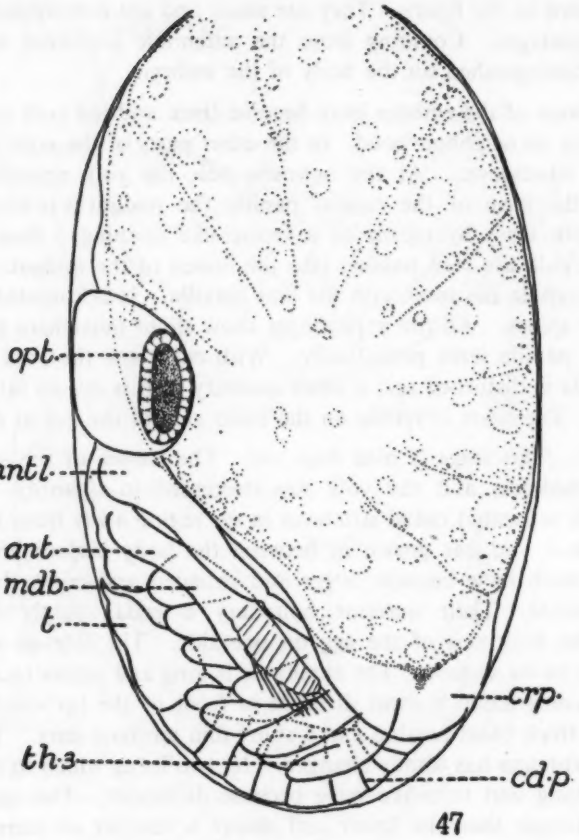


FIG. 47

them. The heart begins to pulsate about this stage. Slight variations have been observed in the commencement of the pulsation. In some it starts as much as six hours earlier than this stage, in some others three to four hours later. When it comes to its full activity it beats about 150 times per minute. As the heart lies at the base of the caudal papilla immediately above the proximal end of the midgut rudiment with which it is in contact these pulsations help greatly in disturbing the yolk and driving it into the papilla.

Fig. 48. Twelve and a half days old. This is an advanced stage in development. The yolk is considerably reduced in quantity. It has become enclosed on all sides by tissues and appears divided into four rounded blocks

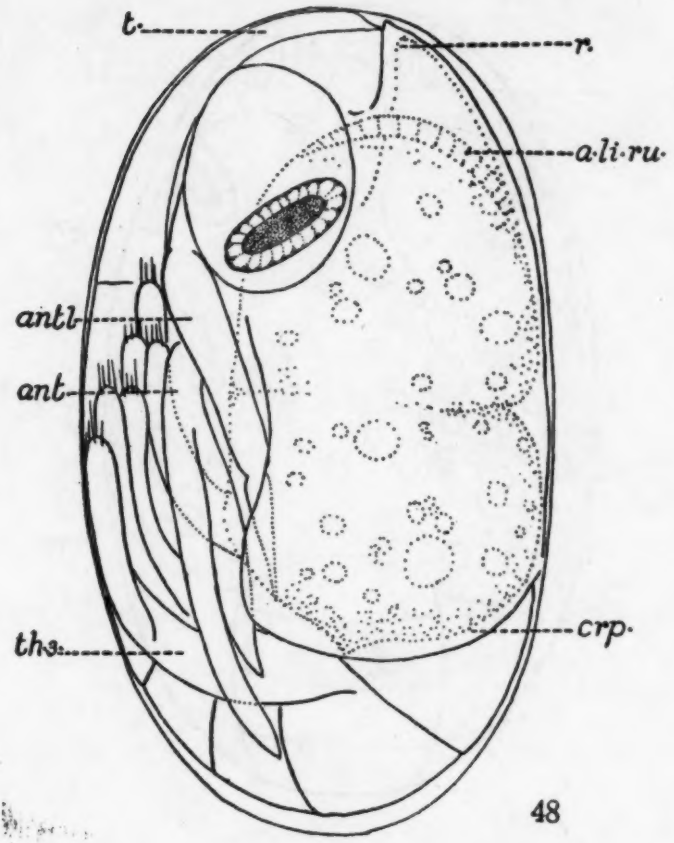


FIG. 48

two anterior and two posterior. The embryo occupies the whole of the long side of the egg. The eyes which have become large and massive lie at the anterior end. The tip of the telson bends round the eye and points upwards reaching almost the dorsal side. Fig. 49 is of the same stage and shows the dorsal view of the embryo. The anterior margin of the carapace is well defined and the rostrum projects forwards between the eyes as a short blunt process. At the base of the rostrum a dark elongated spot is visible. On the front side of the two anterior blocks of yolk a regular layer of endoderm cells has been formed. The yolk in contact with this layer breaks down into a clear fluid in which vacuoles appear. These vacuoles can be seen to gently move. The carapace fold at the posterior end has become

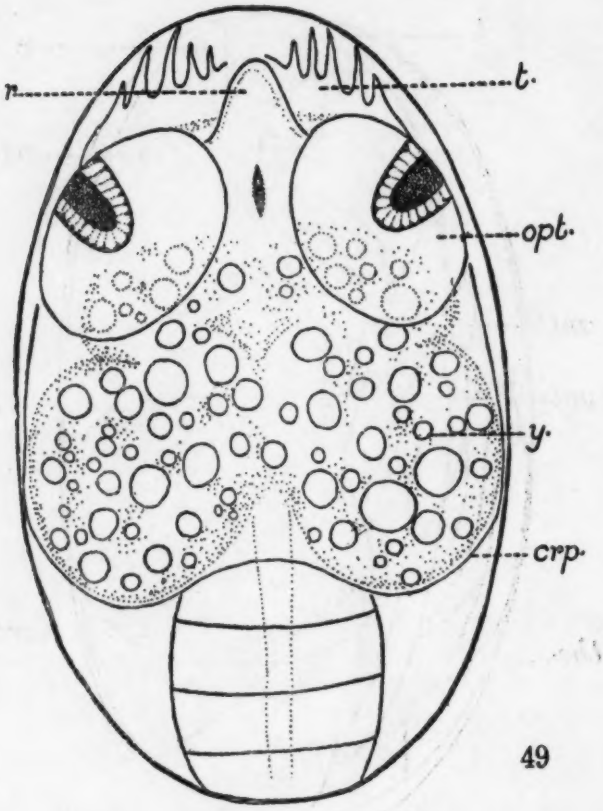
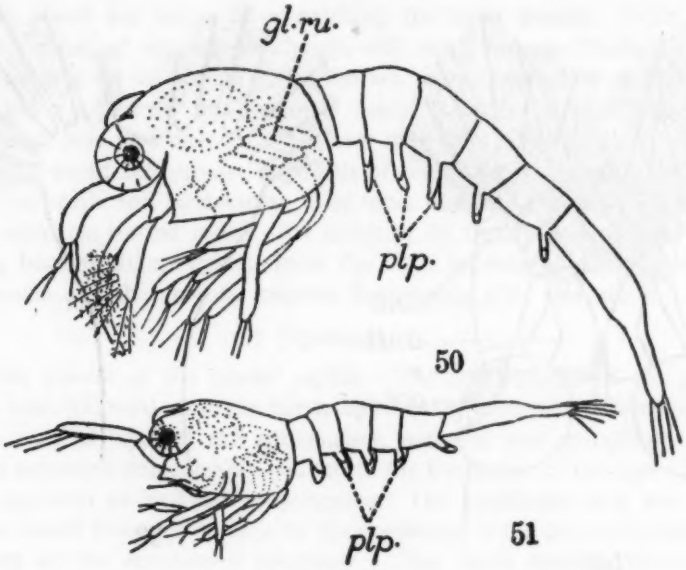


FIG. 49

distinct from the body on either side. The antennule and the antenna are very long; so also are the first three thoracic appendages whose lobes bear setæ at their tips. The succeeding appendages cannot be distinguished in the living embryo inside the envelope, since they are hidden by those in front. But when the embryo is dissected out it is seen that all the eight thoracic appendages have been formed as typical biramous structures, their lobes being finger-shaped. Circulation of fluid is visible inside the body.

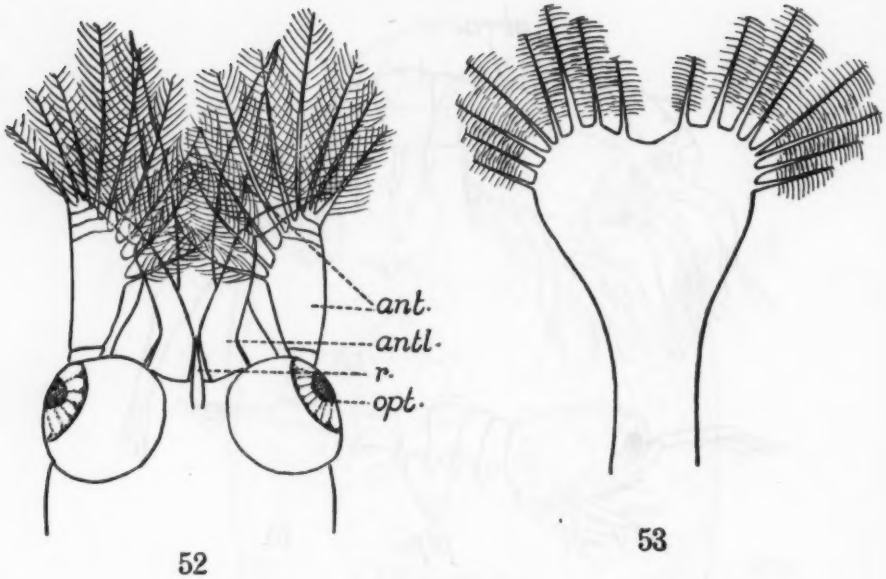
Fig. 50 shows the condition of the embryo sixteen days after the beginning of cleavage. The embryonic envelope has been removed so that the posterior part of the body may stretch out. Evidently the embryo has undergone a moult since the stage described above. There is a small quantity



FIGS. 50 & 51

of yolk inside the gut. The eyes are sessile. In the figure they are shown as projecting outwards, but this condition has been brought about by the tearing out of the embryo from the envelope. In the natural condition they are held back by the pressure of the covering membrane. The antennule and the antenna are also directed forwards. The tip of the former has become bilobed. In the antenna the two lobes are markedly different. The upper one is flat and leaf-like, fringed with plumose setæ, while the lower one is long and tubular tapering to a fine point. The mandible and the two maxillæ are small. All the thoracic appendages are long and biramous. Above the base of the thoracic appendages and below the carapace there appear on the body wall four oblong thickenings lying parallel to one another. These are the rudiments of the gills. The abdomen shows five segments. The last of these is imperceptibly continued into the telson. Each of the first five segments bears a pair of bud-shaped appendages on the ventral side. At this stage these abdominal appendages are not bilobed, but the fact that they are internally becoming so can be made out through the transparent cuticle. The posterior end of the abdomen is long, flat and thin. The telson is broader behind and possesses two rounded lobes fringed with setæ.

Figs. 51, 52, 53 show the newly hatched larva. Hatching takes place at night and is accompanied by the ecdysis of the mother. The larva



FIGS. 52 & 53

measures 2425 microns from the tip of the rostrum to the edge of the telson. The distance from the tip of the eye to the hinder edge of the carapace is 720 microns. The thoracic appendages are blunt and finger-shaped. The first pair is shorter than the rest. The abdominal appendages are still single lobed and the uropods are absent. Each lobe of the telson shows eight spine-like setæ along its posterior margin. A small quantity of yolk remains unabsorbed, but it is all broken up and liquefied. The larva keeps the second maxillæ constantly vibrating. Inside the basal segment of the antenna a coiled duct can be seen through the transparent body wall. This evidently is the antennal gland. The larvæ swim about in the water with a flitting movement much in the manner of water fleas. It frequently comes to rest at the surface where it hangs head downwards, the telson touching the meniscus. It possesses nine chromatophores arranged in the following order.

One on each antennule;
One on the basal segment of each antenna;
One in the region of the mandible and the first maxilla of each side;
One in the region of the first and second thoracic appendages of each side;
One (unpaired) in the last segment of the abdomen at the point of origin of the telson.

Within about ten hours after hatching the larva moults. With this moult the abdominal appendages though still small become bilobed. The uropods continue to be absent. The thoracic appendages lose their blunt finger-shaped appearance and become many jointed. The direction in which they are held also changes. Till now they were all pointing forwards, but after this moult the terminal segments of the posterior thoracic appendages turn outwards and backwards. The larva moults again after 24 hours and only with this second moult after hatching do the uropods appear and the telson become differentiated from the sixth segment of the abdomen. The antennule and the antenna become filamentous after this moult.

(*b*) *Organogeny*

1. *The growth of the caudal papilla.*—The development of the germ layers has been followed up to the late nauplius stage where the differentiation of the ectodermal layer of the antennulary, antennal and mandibular segments had advanced considerably and led to the formation of the appendages of these segments as bud-like prominences. The teloblastic area was seen to become raised from the surface by the deepening of the caudal furrow and the activity of the ectodermal teloblasts. The latter arrange themselves in a circular row around the telson area in which the anus is situated. They are 21 in number. Figs. 54 to 60 represent a series of transverse sections

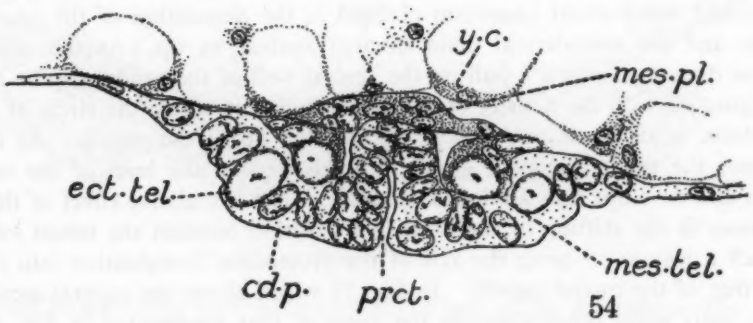


FIG. 54

from behind forwards of an embryo which is slightly older than that represented in Fig. 43. The proctodæum projects vertically upwards against the yolk (Fig. 54). On either side of it, at the edge of the raised area two ectodermal teloblasts could be recognized. They have already formed three descendants each and these lie in the bend between the edge of the teloblastic area and the general surface of the body, which is actually the lateral extension of the caudal furrow formed as an arched groove in front. The three

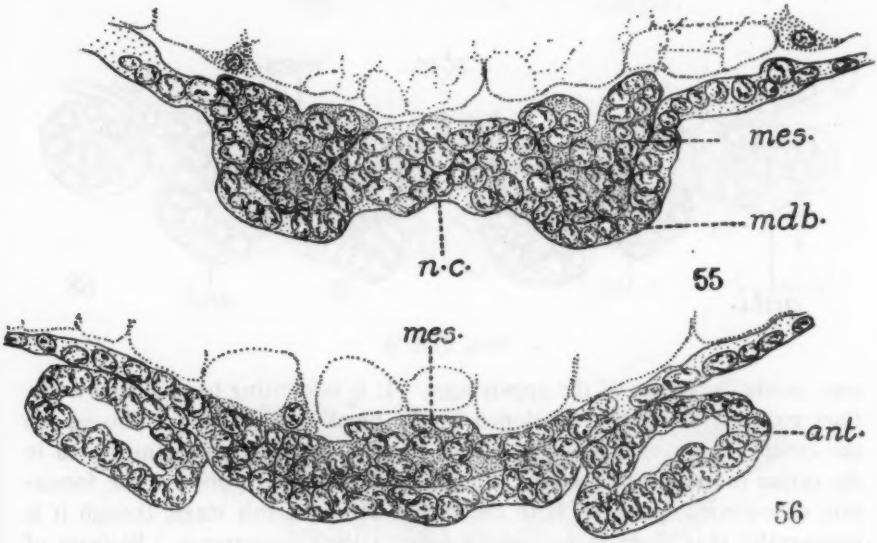
rows of teloblastic descendants represent the elements which ultimately form the segments coming immediately behind the mandible. This differentiation into segments is hardly in evidence at this stage.

As is well known the growth of the posterior region of the embryo takes place by the activity of the teloblasts both ectodermal and mesodermal. The descendants of the former give rise to the segmental ectoderm and also the rudiments of the appendages while those of the latter form the somites. The foundations of the first five post-naupliar segments are laid even before the teloblasts arrange themselves in a complete circle, each segment being composed of one row of teloblastic descendants. As a result of this these segments are represented only by their ventral side just like the naupliar segments. They lie on the main body of the embryo on the ventral side of the yolk mass, while the succeeding segments, which are developed after the arrangement of the teloblasts in a regular circle, are fully formed from the beginning. It is these segments which go to form the caudal papilla that projects from the main body of the embryo. The teloblastic descendants (ectodermal) divide rapidly forming several rows one behind the other and in the ectodermal layer so constituted the segments are marked out externally. The appendages arise as paired hollow bud-like projections from each segment.

The raising of the teloblastic area and the growth of the caudal papilla so formed bring about important changes in the disposition of the proctodæum and the mesodermal plate situated against its tip. As the caudal furrow deepens it exerts a pull on the ventral wall of the caudal papilla and this, coupled with the activity of the telson rudiment within the circle of the teloblasts, tends to draw the anus towards the tip of the papilla. As this happens the telson rudiment grows forwards beyond the level of the teloblasts and develops into a bilobed structure. The cumulative effect of these processes is the shifting of the anus to a position between the telson lobes in such a way as to bring the axis of the proctodæal invagination into line with that of the caudal papilla. In Fig. 71 which shows the sagittal section of an early stage (approximately the same as that represented in Fig. 44) the anus is still on the dorsal side of the papilla. In Fig. 72 which is a parasagittal section of a later stage the terminal shift is in progress. By the time the stage represented in Fig. 45 is reached the anus comes to occupy a terminal position between the lobes of the telson. But even here it is situated nearer the dorsal side than the ventral. In Fig. 73 which is of a still later stage it is fast moving towards the ventral side. One important feature that is noticeable in these series of sections is that the proctodæal invagination has not appreciably grown during the course of the changes just described.

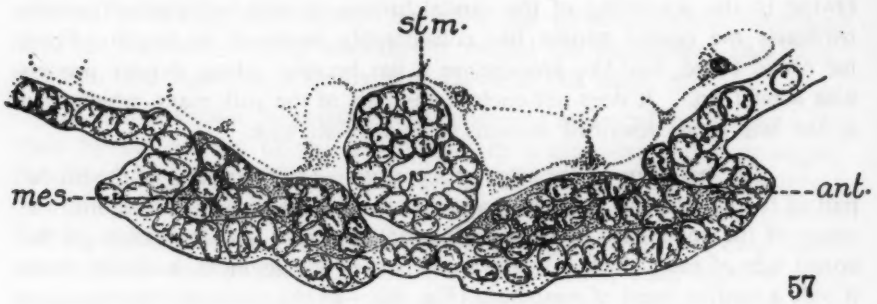
Owing to the deepening of the caudal furrow, as also to the activity of the teloblasts the caudal papilla has considerably increased in length. From the broad based, bud-like prominence it has become a long slender process with a bifid tip. It does not enclose any part of the yolk mass, which even at the late stage described remains level with its base.

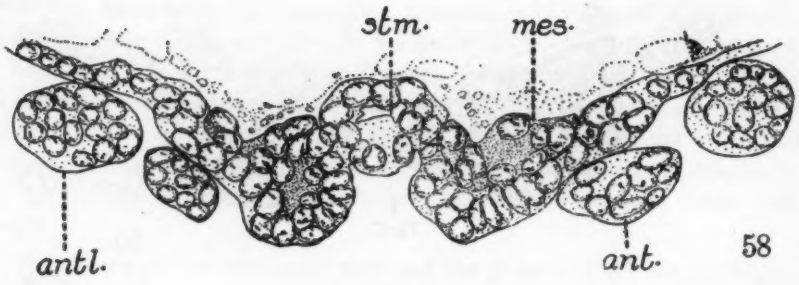
2. *The mesoderm of the naupliar segments.*—The mesoderm of the hinder part of the mandibular segment grows into two blocks each projecting into the cavity of the appendage of its side (Fig. 55). There is a depression on the dorsal side of each block. In the front part of the segment, however, there is only a median band of mesoderm (Fig. 56). In the antennal segment there



FIGS. 55 & 56

are two broad blocks each lying against the base of the appendage (Fig. 57). The stomodæum projects back from the mouth and consequently in the section shown it has been cut transversely a short distance behind the mouth. Fig. 58 shows a section passing through the region of the mouth. The mesoderm of the antennal segment descends into the ectodermal fold on either side of the mouth. Fig. 59 and Fig. 60 show sections passing through the antennulary segment. The mesoderm appears as two distinct rounded blocks here, each lying a short distance away from the point of origin of the appendage and towards the median line. Stray mesoderm cells can be

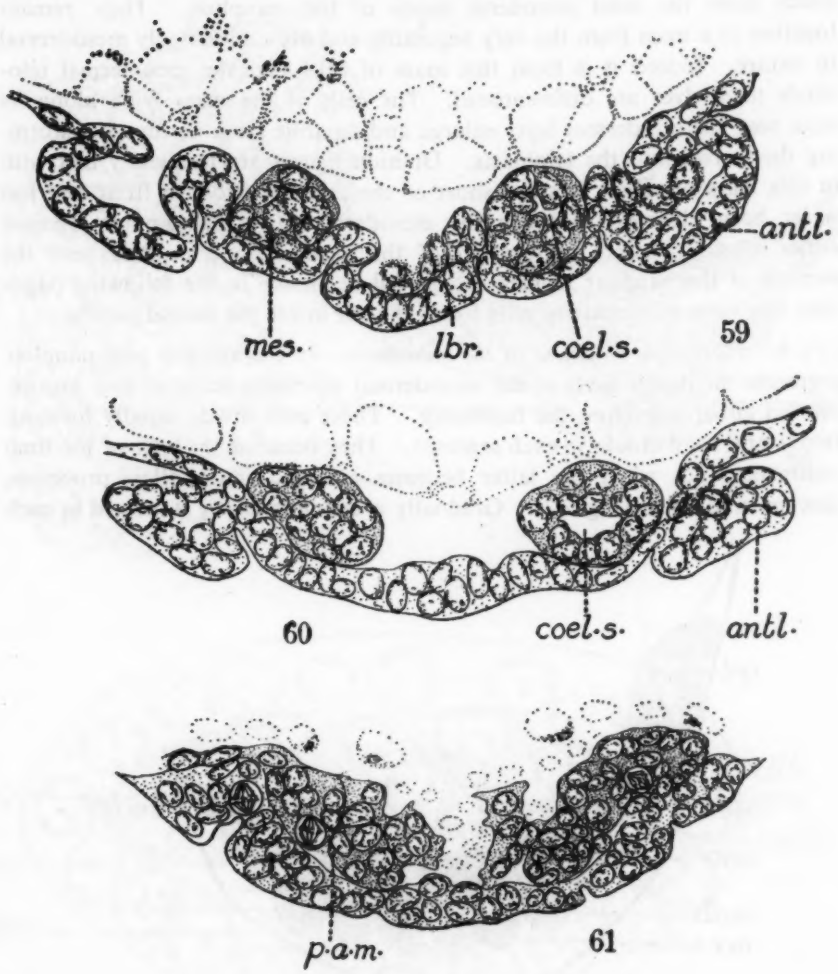




FIGS. 57 & 58

seen inside the cavity of the appendages. It is interesting to note that a distinct cœlomic space has developed in the block on one side. Though on the other side no clear space is visible there is a less deeply stained area in the centre in one of the sections. It has been possible to observe the formation of cœlomic spaces in both the blocks at about this stage, though it is noteworthy that there is no simultaneity in their appearance. Sections of the region in front of the antennulary segment show that the pre-antennulary mesoderm blocks have grown considerably in size and are extending towards the median line (Fig. 61). No cavity has yet been differentiated in these mesoderm blocks.

3. *The mesodermal teloblasts.*—The mesodermal teloblasts arrange themselves in a circular row around the proctodæum at about the stage represented in Fig. 43. The circle is smaller than that formed by the ectodermal teloblasts. They are also fewer in number, probably 10 or 12. At the stage shown in Fig. 54 they have only divided twice producing two rows of descendants which lie towards the yolk mass. The tip of the proctodæum juts against a flattened mass of darkly staining cells with small nuclei.



FIGS. 59–61

This lies below the row of large yolk cells at the periphery of the yolk mass. This mass of cells is clearly demarcated from the descendants of the mesodermal teloblasts with which it is in contact. It is thicker in the central region and becomes thinner towards the edge. The history of this mass of cells has been closely and carefully followed. It is composed of cells which have been budded off from the blastopore at the end of gastrulation and which have moved towards the teloblastic area close behind the elements

which form the head mesoderm bands of the nauplius. They remain together in a mass from the very beginning and are undoubtedly mesodermal in nature. Indeed it is from this mass of cells that the mesodermal teloblasts themselves are differentiated. The cells of the mass lying along its edge near the ectodermal layer enlarge and separate from it, thus transforming themselves into the teloblasts. Division figures are frequently met with in this mass of cells. It stops short of the caudal furrow in front and has never been observed to touch the mesoderm of the mandibular segment either after or before segregation of the head mesoderm bands into the somites of the naupliar segments. It will be shown in the following pages that this mass of mesoderm cells forms the gut inside the caudal papilla.

4. *Further development of the mesoderm.*—In the anterior post-naupliar segments the descendants of the mesodermal teloblasts occur in two groups one on either side from the beginning. These cells divide rapidly forming two lateral solid blocks in each segment. They occur at the base of the limb rudiments and, when the latter becomes enlarged into hollow processes, descend into them (Fig. 62). Gradually a cœlomic cavity is formed in each

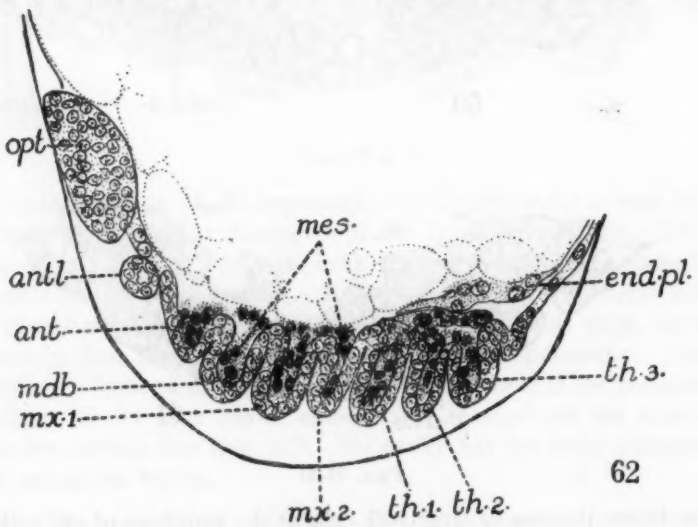
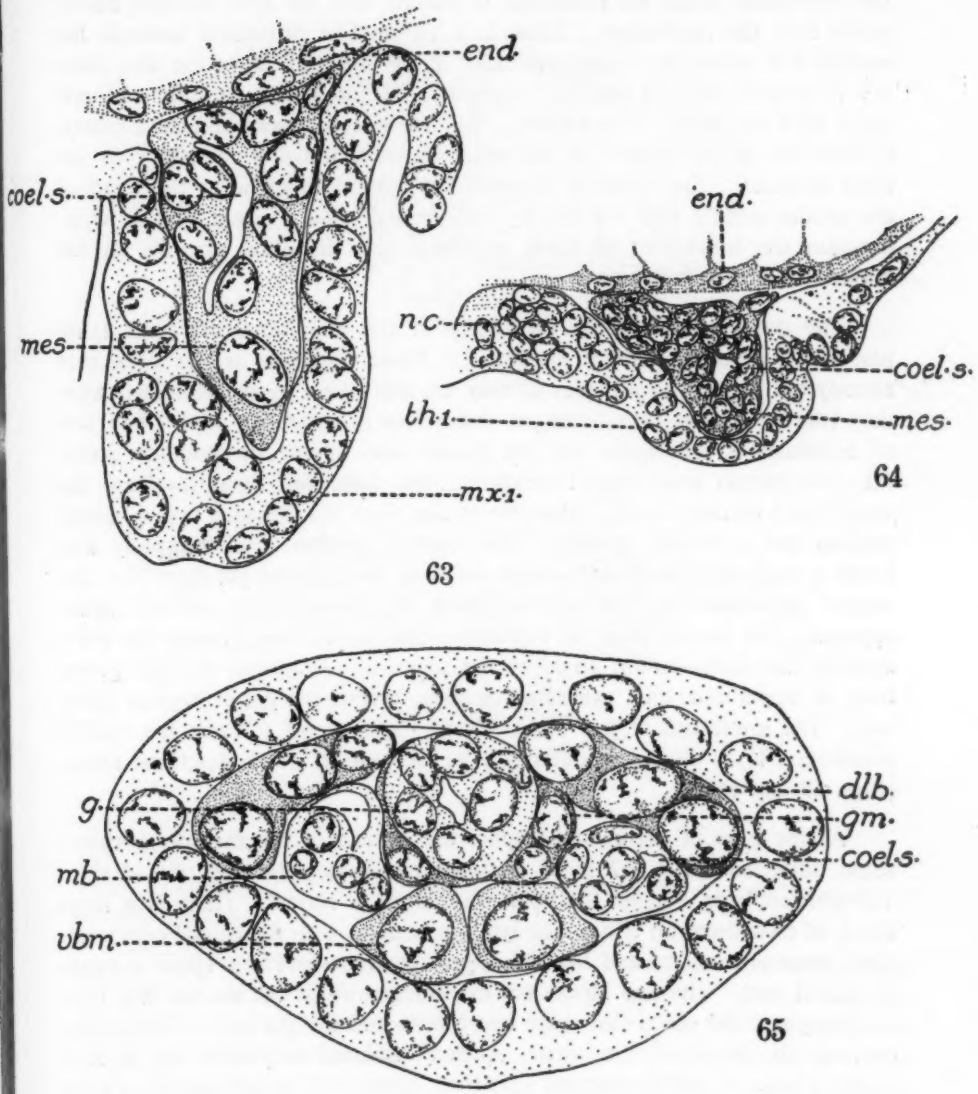


FIG. 62

of these blocks. The cavities are narrow in the front-to-back direction and they bend towards the front at the upper end (Fig. 63). By the time cœlomic spaces appear in the anterior segments the ectoderm in the mid-ventral line becomes thickened. The thickening projects upwards between the



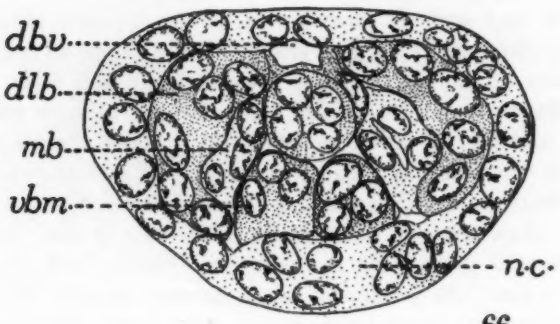
FIGS. 63–65

mesoderm blocks and almost touch the yolk mass above (Fig. 64). It is developing into the ventral chain of nerve ganglia. In transverse section

the mesoderm blocks are triangular in outline with the apex directed downwards into the appendage. There is a tongue-like projection towards the median line where the nerve cord lies. In the opposite direction also there is a projection but it is smaller. Viewed in the transverse plane the cœlomic space does not appear very narrow. The mesoderm blocks and the cœlomic cavities are clearly formed in all segments from the first maxillary to the third thoracic. The segments immediately behind these lie in the bend of the caudal papilla and are greatly compressed. As a result of this compression the mesoderm of these segments gets displaced dorsally in the manner described below.

The development of the mesoderm of the abdominal segments takes place in a different manner altogether. These segments as has been said already are formed on all sides as they are laid down by the teloblasts after they (the teloblasts) have arranged themselves in a circular row. The row of teloblasts is incomplete on the dorsal side. Their descendants divide into two blocks soon after formation. One large cell separates from the ventral end of each block. The rest of the block divides into two, a dorsal portion and a ventral portion. The ventral portion divides rapidly and forms a number of small cells which move up towards the gut from near the ventral ectoderm. In this ventral block of mesoderm a cœlomic space appears. The dorsal block of mesoderm remains in contact with the ectoderm at the dorso-lateral angle of the segment. A tongue of cells grows from its upper end, and, extending inwards, invests the gut at regular intervals. The lower end of this block of mesoderm grows towards the ventral ectodermal layer along the outer side of the ventral block described above (Figs. 65 and 66).

At the base of the caudal papilla the growth of the mesoderm shows some difference. Fig. 67 is of a transverse section through this region. The portion lying outside the yolk mass alone is shown. There is a large block of mesoderm on either side lying in contact with the dorsal ectoderm. Each mass is rounded and has developed a narrow cœlomic space towards its lateral part. The two blocks are extending towards the median line over the midgut. The space that these two blocks and the gut enclose ultimately becomes the lumen of the heart. Already, isolated corpuscles can be seen in this place. It will be realised that these mesoderm blocks have also been formed from the descendants of the circular row of mesodermal teloblasts; but, since this segment occurs at the bend of the caudal papilla which is subjected to considerable pressure, these cells have moved dorsally along either side and have become situated in the position represented in the



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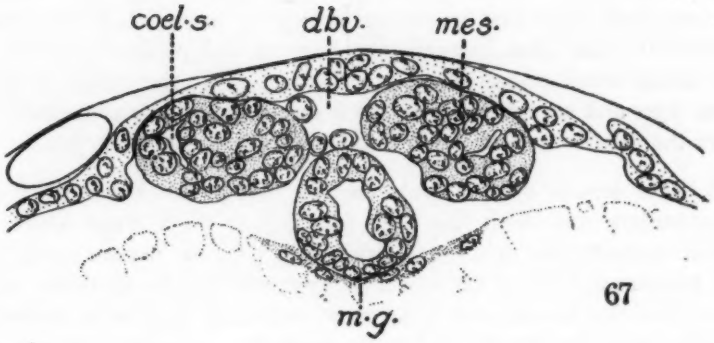


67

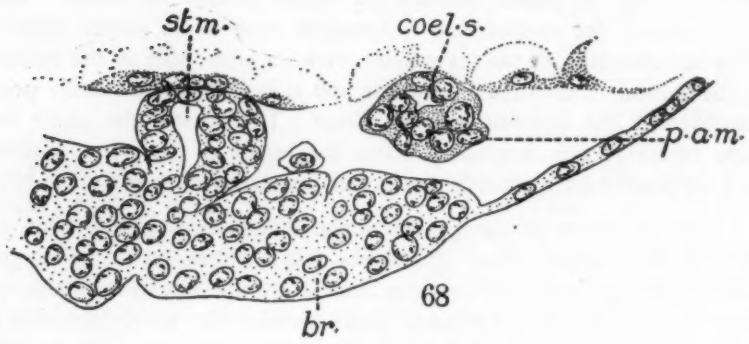
FIGS. 66 & 67

figure. There is little if any mesoderm at the ventral side of the segments of this region.

The later development of the mesoderm is extremely complicated and has been traced only in broad outline. In the anterior thoracic segments the dorsal portions of the somites grow upwards between the yolk mass and the ectodermal layer and as they do so get converted into muscles (Fig. 78). This differentiation also takes place in the part of the somite extending horizontally towards the ventral nerve cord. The cœlomic spaces in these somites persist for a long time, even after the differentiation of their walls into muscles has taken place. Very few mesodermal cells or their derivatives can be distinguished between the yolk mass and the ectodermal layer on the dorsal side of the body even in the advanced stages of the embryo.

Inside the caudal papilla the two large cells lying on either side of the mid-ventral line in each segment remain without any apparent change for some time, but later divide and form two groups of cells (Figs. 65 and 66). These two cell masses do not show any connection with the other mesodermal elements inside the body. It is from them that the massive ventral musculature of the abdomen is developed (Fig. 79). Above these are found two other blocks in which, as has already been described, cœlomic cavities appear, and beyond them, in the dorso-lateral region, a row of cells. The middle blocks do not show any elaborate growth or increase in size. Each of these blocks gets pressed in between the other two blocks both of which grow considerably in size. As a result of this it becomes thinner and thinner and ultimately the cœlomic space inside it gets obliterated. Its lower end gains an attachment to the ventral ectodermal layer. This attachment is very much restricted in area. Its upper end also becomes attached to the dorso-lateral mesodermal blocks at the point where the tongue-like extensions grow out from them and invest the alimentary canal. It is this strand of mesodermal tissue that becomes the base of the pericardial floor. The dorso-lateral block of mesoderm grows down along the inside of the ectodermal layer and becomes attached to it on its ventral aspect in front of and behind the insertion of the base of the pericardial floor (Fig. 80).

5. *The growth of the preantennulary mesoderm.*—The growth of the preantennulary mesoderm has been followed up to a stage where it has extended considerably in size and lies in two irregular masses on either side some distance in front of the labrum. The mass of cells on either side now divides into two blocks, one lying more anteriorly and more laterally and the other more posteriorly and more medially. A portion of one of the former blocks is seen in Fig. 68. A cœlomic space has already appeared in it. The latter blocks are smaller than the former. They move backwards, close to the ectoderm, into the labrum along either side of the stomodæum which by now has grown forwards (Fig. 69). A cœlomic cavity appears in this block. The other two blocks which are larger and more dorsal in position also move backwards, but this happens along the medial plane and, as they do so, come into contact with the anterior face of the stomodæum. Gradually they apply themselves to the wall of the stomodæum and their hinder portions form an investment over it (Fig. 69). The cavities in these are confined to the anterior part of each block and do not extend into the part which invests the stomodæum. This part, as soon as it comes in contact with the stomodæum, meets with its fellow of the other side and forms a continuous layer. This layer of cells gradually grows back over the stomodæum and envelopes it completely on all sides. The transverse section



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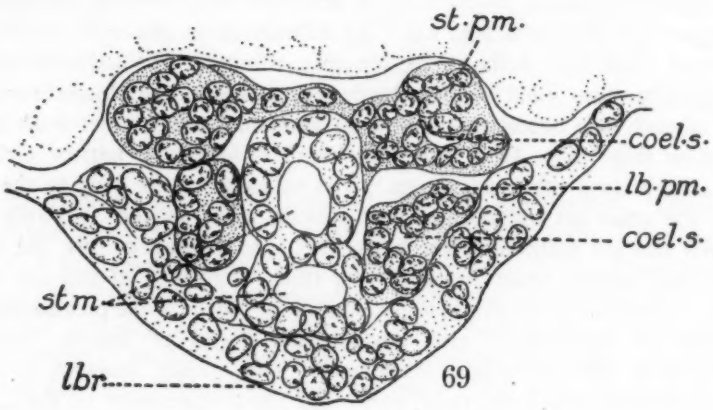

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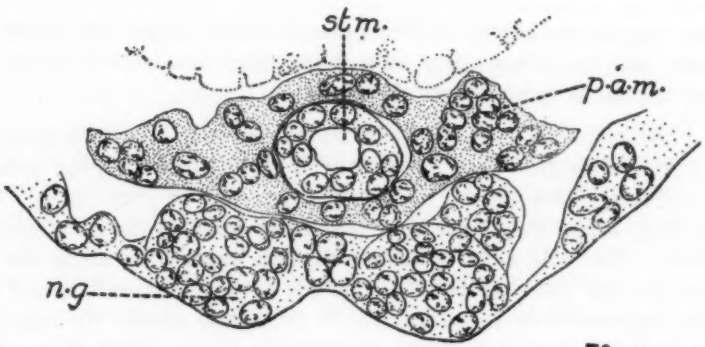

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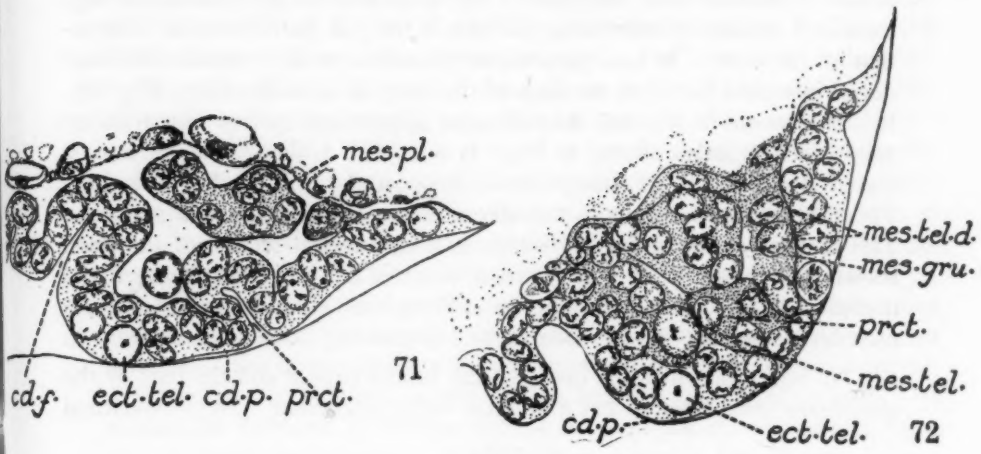
FIGS. 68–70

shown in Fig. 70 passes through the region behind the mouth. Here the investment of the stomodæum is complete even in its hinder aspect. The wing-like extension of the investment, seen on either side of the stomodæum in the section, is continued forwards and it is in these forwardly projecting regions that the cœlomic cavities occur. The blastocœlic space between these two blocks is destined to form the lumen of the anterior aorta and here corpuscles have already made their appearance at this stage (Fig. 68).

Differentiation of the preantennulary mesoderm blocks into muscles starts on their dorsal side. The blocks that are attached to the stomodæum and project outwards and forwards from it are the first to show this change. They lie close behind the brain and the muscles that are differentiated from their upper ends attach to the fold of ectoderm that grows in between the yolk mass and the eyes (Fig. 81). As differentiation proceeds further the investment over the stomodæum becomes thinner. The cœlomic spaces in the blocks move back and gradually extend round the stomodæum, the cavity of each side becoming continuous with that of the other both above and below (Fig. 82). Thus ultimately there is formed a narrow cœlomic space surrounding the stomodæum. The mesoderm cells lying to the outside of the space get transformed into muscles which extend forwards and outwards and get inserted on the body wall. As a result of this the outer wall of the cœlomic space becomes very thin, composed of just one layer of cells. The rest of the two massive stomodæal blocks of the preantennulary mesoderm is transformed into muscles. A strand of muscle stretches dorsally from the stomodæal investment. This passes upwards between the two anterior liver lobes (*vide* infra) and finds attachment to the dorsal body wall.

The two lower blocks of the preantennulary mesoderm which have descended into the labrum very soon differentiate into muscles there. The cœlomic spaces in these blocks disappear.

6. *The endoderm and the formation of the gut.*—The yolk-laden cells which sink into the yolk mass at the time of gastrulation form the endoderm of the embryo. They absorb more and more yolk as they go in. It has already been stated that these cells frequently undergo division inside the yolk mass. The yolk cells ultimately arrange themselves along the periphery of the yolk mass where they condense to form the endodermal layer. The first condensations to appear are in the region around the base of the caudal papilla. Here a posterior endodermal plate is formed above the flattened mass of mesoderm cells lying against the tip of the proctodæal invagination (Figs. 54 and 72). On either side of the posterior endodermal

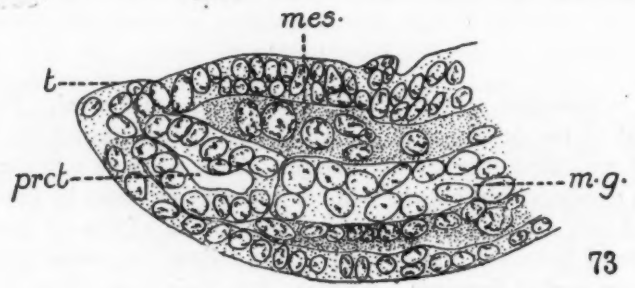


FIGS. 71 & 72

plate two independent condensations of yolk cells are formed. These also are situated at the periphery of the yolk mass and lie against the bases of the second and third thoracic appendages. Each of these consists only of a few cells to begin with, but the number gradually increases by the addition of modified yolk cells along the margin. As a result of this an endodermal plate is formed in each of these places. They follow the contour of the yolk mass and are arched forwards. As they grow their inner edges come into contact with the posterior endodermal plate lying in between and ultimately become continuous with it. These two endodermal structures are evidently the rudiments of the posterior liver lobes. In the anterior region against the tip of the stomodæum condensation of yolk cells takes place and an endodermal plate is formed (Fig. 68). But this lies in contact with the stomodæum, unlike the posterior endodermal plate which is separated from the tip of the proctodæum by the mass of mesoderm cells already described. Subsequently on either side of the anterior endodermal plate two other condensations of yolk cells appear. They grow into plates, following the contour of the yolk mass, and thus getting arched with the concavities facing back towards the centre of the body of the embryo. Between these two cup-shaped endodermal rudiments the muscle strands that develop from the mid-dorsal region of the mesodermal investment over the stomodæum pass upwards. In appearance these two rudiments are exactly like the liver rudiments at the back of the yolk mass. They can be distinguished even in the living embryo (Fig. 48). Obviously these structures represent

a pair of anterior liver rudiments. The mesoderm of the mandibular segment as it extends dorsally along the sides of the yolk mass causes an indentation on the latter. In later growth two prominent muscle strands with their dorsal insertions far up at the sides of the body are developed here (Fig. 85). It is on account of this that the yolk mass appears cut up into four rounded masses at the stages shown in Figs. 48 and 49. A similar stouter muscle strand is developed on either side at the posterior end of the yolk mass. These are formed from the mesoderm of the anterior thoracic segments. This is clearly seen in Fig. 85 which is a parasagittal section of the stage shown in Fig. 48. The anterior-most band of muscles seen in the figure immediately behind the eye is the one differentiated from the preantennulary mesoderm investing the stomodæum and proceeding dorso-laterally from it.

No yolk cells enter the cavity of the caudal papilla and the part of the alimentary canal inside it has a different origin altogether. The mesodermal



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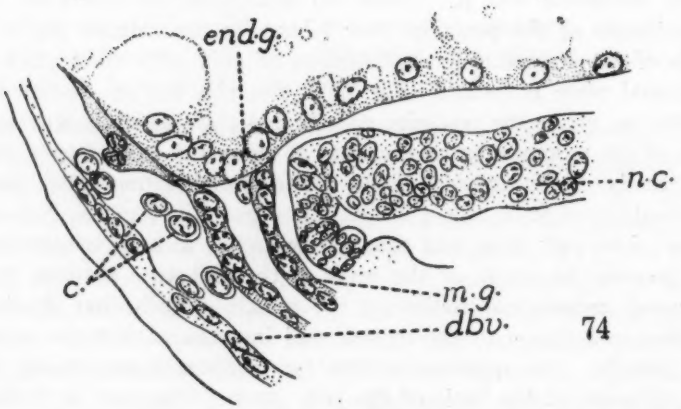


74

FIGS. 73 & 74

mass that lies against the tip of the proctodæum undergoes marked changes with the growth of the caudal papilla. Division figures can be frequently distinguished in these cells, showing that they are increasing in number. As the yolk cells that lie above it along the periphery of the yolk mass (Fig. 54) condense into the posterior endodermal plate these cells become a rounded solid mass (Fig. 72). With the increase in length of the caudal papilla the rounded mass grows into a rod of cells lying parallel to the axis of the papilla more towards its dorsal side (Fig. 73). At the stage represented in Fig. 73 no cavity could be distinguished in it. But by the time the embryo reaches the stage shown in Fig. 46 a cavity appears and the structure becomes an elongated tube. This tube is wider at the proximal end of the caudal papilla and, to start with, it is actually closed by the endodermal plate at this point (Fig. 74). The latter structure has increased considerably in extent by this time and is not confined merely to the base of the caudal papilla. It extends far forwards in the direction of the head region. Posteriorly, however, it is very much limited. The condition shown in Fig. 74 does not last

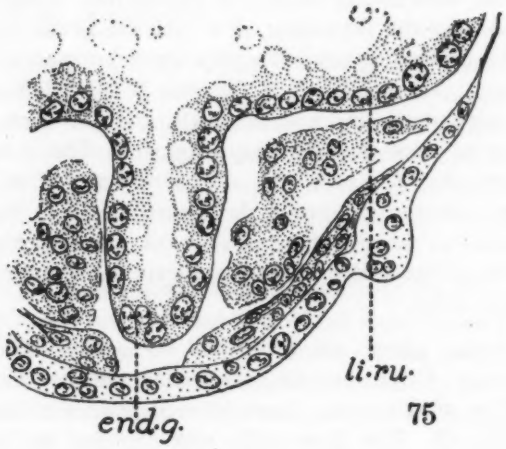


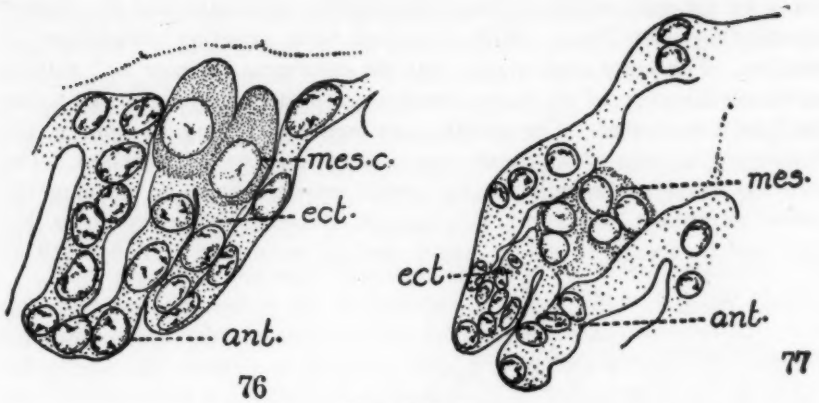
FIG. 75

very long. Very soon communication is established between the mesodermal tube and the yolk sac. In spite of repeated attempts it has not been possible to observe how exactly this is effected. The embryo begins to show the first signs of movement at about this stage and it has already been stated that these movements are particularly noticeable in the caudal papilla which jerks frequently. Perhaps these movements are responsible for breaking the endodermal plate at the point where it covers the proximal end of the

mesodermal gut tube. In Fig. 74 the plate is actually dipping down at this point. Whatever the cause, communication is established and the liquefied yolk that occurs in and around this region passes into the tube. With this the mass of yolk begins to shrink rapidly. The yolk cells occurring along its periphery condense into the midgut epithelium and the alimentary canal is thus completed.

It will be clear from the account given above that the part of the gut of *Caridina* lying immediately in front of the proctodæum is mesodermal in origin. It extends from the tip of the proctodæum to the point where the caudal papilla is bent over, *viz.*, the third thoracic segment. Only in front of this point does the endodermal layer enter into the formation of the alimentary canal. Sollaud (1923) has pointed out the mixed origin of the midgut in the Palæmoninæ, but his evidence for such an inference has largely been circumstantial. This, naturally, has not succeeded in convincing later workers on Crustacean embryology, particularly because of the fact that the differentiation of the germ layers in the Palæmoninæ, as described by Sollaud, is not so determinate as in the Peracarida. Gastrulation in the former group leads to the formation of a mesendodermal mass which lies at the base of the developing caudal papilla and takes part in the organisation of the midgut. The condition in *Caridina* is quite different. Here the development is very much more determinate and the germ elements are completely separated from the time they originate. The mass of cells that comes to lie below the teloblastic area is mesodermal and this by sustained growth developes into the gut inside the caudal papilla. The endodermal plate that is formed at the periphery of the yolk mass in this region has a different origin altogether and it remains separate from the mesodermal gut rudiment till a late stage.

7. *The antennal gland, the brain and the gonad.*—The observations on the development of the antennal gland relate only to its early phases. The first indication of this organ becomes recognizable at about the stage represented in Fig. 45. Two large cells with rounded nuclei can be seen lying at the base of the antenna against its posterior aspect. These cells evidently are of mesodermal origin as they lie within the cavity of the appendage. Gradually the ectoderm of the wall of the appendage gets invaginated at this spot. The tip of the invagination comes in contact with the two cells mentioned above and pushes them inwards (Fig. 76). This is the condition observed in the embryo which has reached the stage represented in Fig. 47. Only one other step in the development of the gland has been observed. This is the division of the two large mesodermal cells to form a group of cells. This condition is reached when the embryo is at



FIGS. 76 & 77

the stage shown in Fig. 48. No other structure is seen inside the base of the antenna near these rudiments. It would appear that the antennal gland lags behind a little in development.

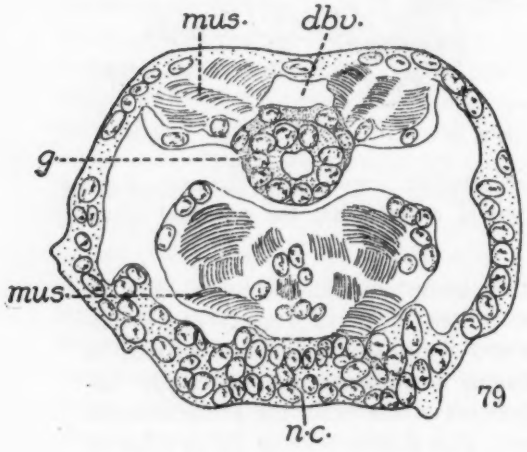
A word may also be said about the composition of the brain. Fig. 81 shows a slightly parasagittal section of an advanced stage. The protocerebrum, the antennulary ganglion and the antennal ganglion as well as the beginning of the circumoesophageal commissure can be clearly distinguished in it. Of the three components of the brain the protocerebrum is very much larger than the other two. It is almost twice as big as either of the other two ganglia.

The gonad arises as a paired rudiment very near the base of the caudal papilla. The rudiments are on either side of the mesodermal gut. They are formed by proliferation from the ventral surface of the pericardial floor and consist of large clear cells with conspicuous rounded nuclei (Fig. 83). The rudiments are small and extend only through three consecutive sections.

DISCUSSION

The egg of *Caridina* resembles that of the large majority of the higher crustacea in being typically centrolecithal. Though the quantity of yolk is very large, its disposition in respect of the zygote nucleus agrees with what has been described in a number of forms belonging to the Caridea and otherwise. This egg, however, possesses only a single envelope and it is not quite clear whether this is the chorion or the vitelline membrane. Ischikawa (1885) has shown that the egg of *Xiphocaridina* possesses two envelopes, one formed by the peripheral layer of the egg itself and the other secreted

over it by the walls of the oviduct—the vitelline membrane and the chorion respectively—while Terao (1929) describes three covering membranes in *Panulirus*. The latter author says that the outermost of these is " nothing but the continuation of the gluey thread or cord that attaches the egg to the setæ " of the mother. The middle membrane, according to him, is the chorion. It is very conspicuous measuring 2 microns in thickness. The innermost envelope he calls the embryonic exuvia, obviously meaning the vitelline membrane. Sollaud (1923) has not devoted much attention to this point, and he speaks of only a single vitelline membrane. Yonge (1938)



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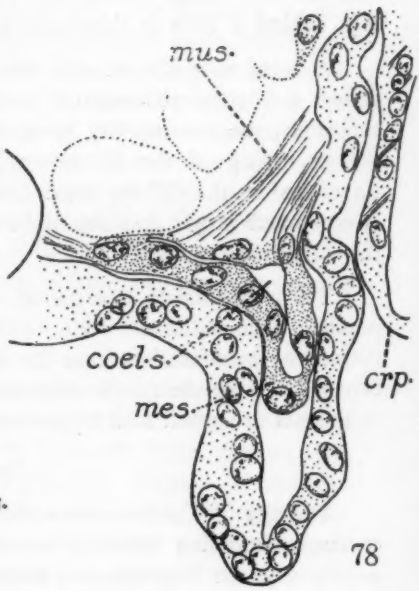

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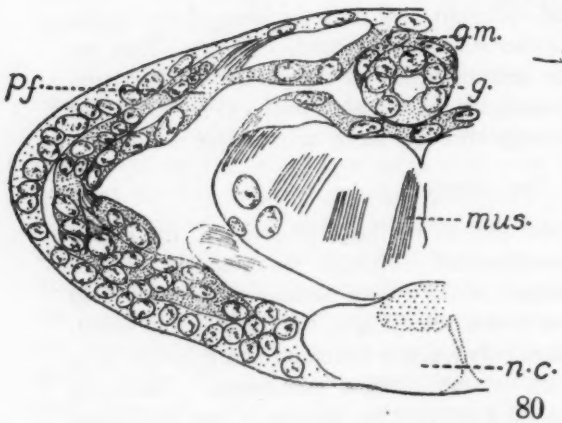

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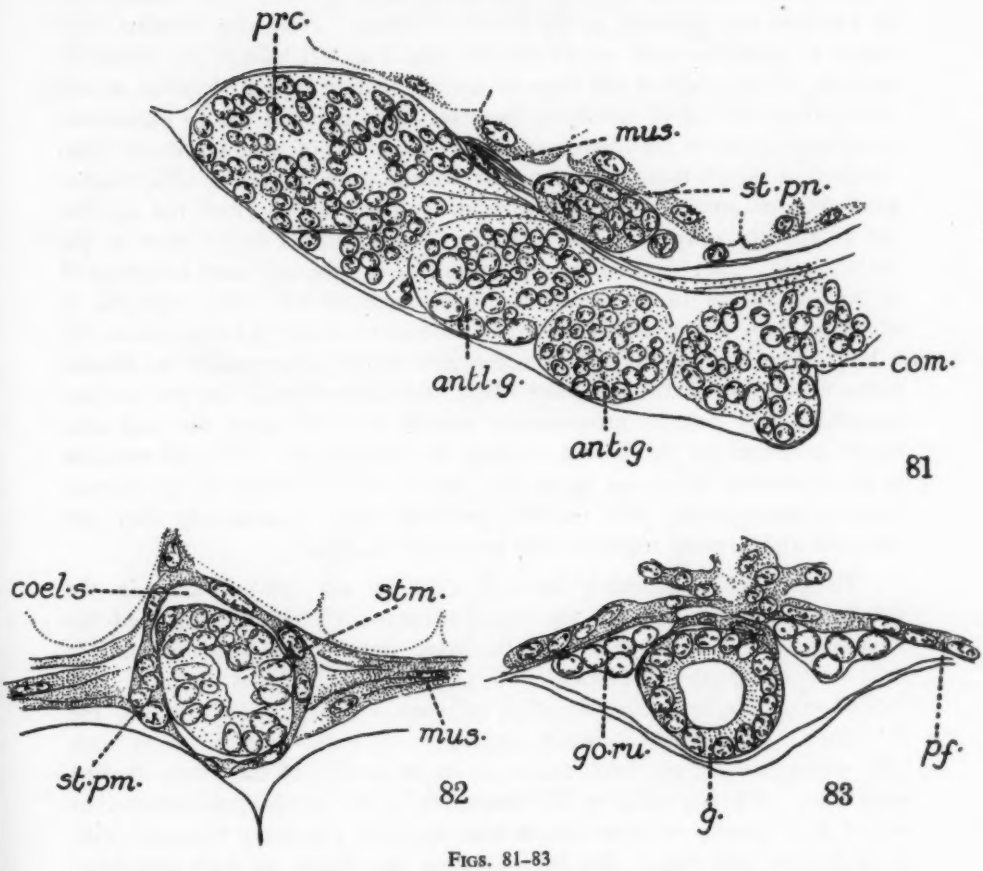
FIGS. 78–80

subjected the ovarian envelopes of *Homarus vulgaris* to a detailed study and came to the conclusion that of the two envelopes in this form the inner one is chitinous and is secreted by the oviduct, while the outer one is composed of the secretion of the cement glands. It is not known whether these conclusions will hold good for other forms as well. However the significance of the differences regarding the number of ovarian envelopes exhibited by the Decapoda is far from clear. The usual number would appear to be two, though exceptions are not uncommon.

With regard to cleavage, *Caridina* exhibits many striking features. The divisions are total up to the 16-blastomere stage. Not only are cleavage furrows passing right through the yolk formed, but the blastomeres also round off giving the embryo a characteristic grape-bunch appearance. Among the Caridea such a condition has previously been recorded only in *Xiphocaridina* (Ischikawa, 1885). Total cleavage has, of course, been claimed to occur in a few other forms as well but in none of these does the rounding off of the blastomeres that we see in *Xiphocaridina* and *Caridina* take place. Mention may here be made of *Virbius* studied by Gorham (1895). Here the eggs are comparatively small measuring only 0·36 mm. The first, second, third and fourth divisions are total involving the entire quantity of yolk. Then there is the doubtful case of *Leander adspersus* where Bobretsky (quoted by Sollaud, 1923) has maintained that cleavage is regular and total in the first stages. It is well known that in *Lucifer* cleavage is not only holoblastic but also leads to the formation of a hollow blastula (Brooks, 1882). This, however, is exceptional and is certainly brought about by the fact that in the eggs of this form there is very little yolk. In *Crangon* (Kingsley, 1887; Welden, 1892), *Homarus* (Bumpus, 1891), *Palæmonetes* (Faxom, 1879) and *Leander* (Sollaud, 1923) cleavage affects only part of the yolk near the surface. The furrows do not extend to the centre and meet there. There is always, in these forms, a mass of undivided yolk at the centre. In *Xiphocaridina* the furrows that result from the first and second cleavages close up after a while rendering the surface of the egg smooth. Indeed the first cleavage furrow undergoes this transformation thrice. It disappears and appears again before the onset of the second cleavage. It disappears once again along with the second (longitudinal) furrow and reappears with it after a time. These changes do not occur in *Caridina*. There is also a considerable amount of difference between the two forms in regard to the time intervals between the divisions. In *Xiphocaridina* there is a marked period of rest after the first and second divisions which between them take as much as eight hours for completion. In *Caridina* on the other hand once they start the divisions proceed in regular succession

without any period of rest. Each division takes from thirty to forty minutes for completion and the next division follows immediately.

Sollaud (1923) has pointed out the difficulty of including the various kinds of cleavage met with in the Crustacea under the general headings set out by Korschelt and Heider (1899). These authors have, for instance, placed *Xiphocaridina* and *Eupagurus* under Type II which is defined as including eggs with total cleavage in the first and superficial cleavage in the later stages. This certainly is difficult to justify. In *Xiphocaridina*, as has already been mentioned, the divisions are total from the beginning while in *Eupagurus* according to the earlier work of Meyer (1877) and also the recent observations of Krainska (1935) they become total only from the third cleavage. The condition of the furrows getting lost in an undivided central mass of yolk as seen in *Palæmonetes* and *Crangon* which are also included in this type is never met with in *Xiphocaridina*, where, even after the nuclei have migrated to the periphery, the blastomeres that are formed are completely demarcated. The yolk that lies at the centre though devoid of nuclei continues nevertheless to show division into blocks. In view of these difficulties it is best not to adhere to the scheme of classification given by these authors. This scheme would also appear to have obscured the value of certain observations and prevented the proper assessment thereof. The statement that their Type II with total cleavage in the first and superficial cleavage in the later stages is very common in the Crustacea has, I believe, tended to distract the attention of later workers, including even Sollaud, from some of the very significant features in the cleavage of *Xiphocaridina*. It is even now generally believed that the type of cleavage is essentially determined by the quantity of yolk present in the egg; that total divisions occur where there is little or no yolk. " In general the egg must divide totally when it contains relatively little yolk " says Sollaud (1923), and he quotes the example of *Virbius*. But in *Xiphocaridina* and *Caridina* we have two forms where the eggs are larger and contain a greater quantity of yolk than those of the majority of the Crustacea and yet which divide in a total manner, at least in the early stages. In most of the forms whose development has been followed the eggs are very much smaller and the cleavage seldom total. The divisions usually affect only the nuclei and the cytoplasmic islands surrounding them. In *Mesopodopsis*, for instance, the eggs measure only 350 microns, less than half the size of the eggs of *Caridina*, but still the yolk is unaffected by the cleavage. So also in *Squilla* whose eggs measure 490 microns. It cannot, therefore, be maintained as a general rule that it is the size of the egg, and the quantity of the yolk therein, that determine the type of cleavage. Indeed the behaviour of the eggs of



FIGS. 81–83

Xiphocaridina and *Caridina* in the early stages looks little different from that of alecithal eggs, judging from the way the grooves appear and the blastomeres are rounded off. *Caridina* also shows distinct interspaces between the blastomeres. It is not known if the consistency of the yolk plays any decisive part in this matter. A comparison of the eggs, live as well as fixed, of *Squilla* and *Caridina* seems to preclude such an inference. The true cause of the differences in the type of cleavage exhibited by the Crustacea appears to be other than purely physical.

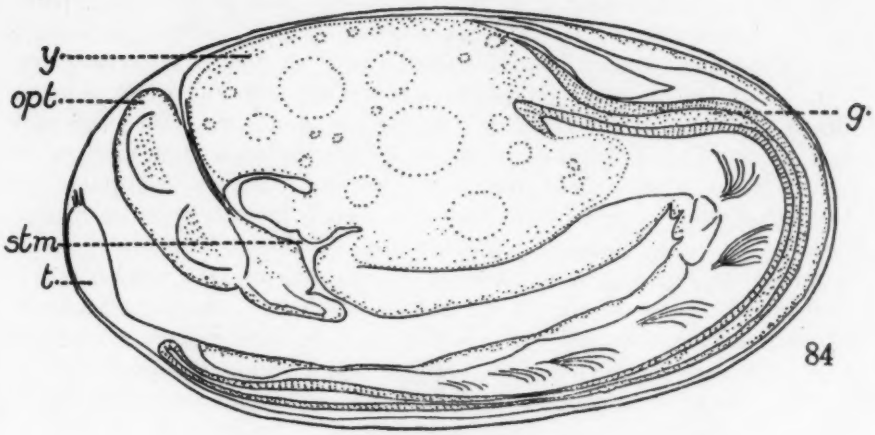
It is customary to call the blastomeres primary yolk pyramids on account of the appearance they display in sections of the early embryo. Though there may be some justification in using this term in the case of forms like *Astacus*

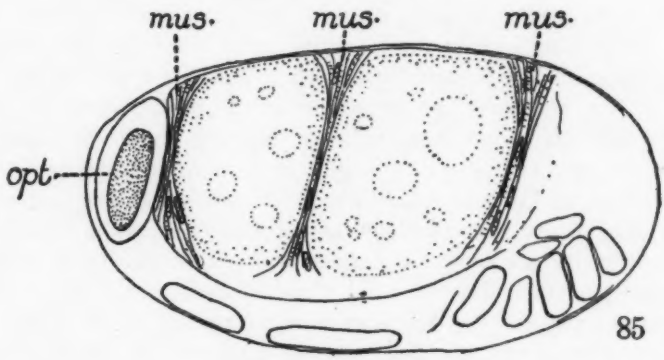
I have deliberately not adopted it in the present account. The blastomeres of *Caridina* are spherical in the living condition; even after fixation their shape is prismatic and not pyramidal (see Fig. 12) though in transverse sections of the embryo the furrows appear to converge to a point at the centre (Fig. 11). This condition, however, lasts only till the 16 blastomere stage beyond which the blastomeres begin to get rid of their burden of yolk. The way in which this takes place has already been described. The blastomeres become smaller with each division for two reasons; one, the division *per se* and the other, the fact that the division affects only a part of the blastomeres. The blastomeres that result from the divisions continue to be surrounded by furrows and also to get rounded off. The yolk that is excluded from the divisions retains its distinctive block-like appearance for a long time. These yolk blocks accumulate in the centre while the blastomeres move towards the periphery where, by a repetition of the process just described, they become progressively smaller and free from the yolk and finally condense on the surface to form the blastoderm. The yolk remains in the segmented condition up to this stage. The roundness of the blastomeres disappears as their number increases and consequently they get crowded and pressed together into polygonal shapes.

There is close similarity between *Caridina* and *Xiphocaridina* in the details of segmentation. In the latter Ischikawa (1885) has described the separation of blocks of yolk from the inner parts of the blastomeres. He calls these non-nucleated blocks " yolk segments " and points out that their formation seems to be an exception to what is generally seen among the Decapoda. His statement would appear to be valid even now, as such yolk segments have not been shown to occur in any of the forms studied since then. What is usual in the Decapoda is for the cleavage furrows to extend to a greater or lesser extent into the yolk and there to merge with an undivided yolk mass. But here, in these two forms, no such undivided central mass remains.

Ischikawa has observed a fair number of nuclei in these yolk segments and considers them as probably derived from the segmentation nulei. Such nuclei do not normally occur in *Caridina* and when they do occur, as rarely happens, their number is very small, not more than two or three. As already stated only two embryos with such nuclei have been observed in the course of this work and I consider their occurrence as accidental.

There is no differentiation of the blastomeres into micromeres and macromeres in *Caridina*. Neither do they show any tendency to condense into the blastoderm on any one particular point of the surface before others. The blastoderm is formed simultaneously all over the surface.





FIGS. 84 & 85

Gastrulation in *Caridina* presents several features which are interesting. It starts from a point which is far removed from the region where the outline of the animal first makes its appearance and which, in view of the orientation of the animal in later development, may even be considered as dorsal in position. It is a spatially elongated process, culminating in the formation of a definitive blastopore which too lies outside the embryonic area. Its position though dorsal with reference to the future orientation of the animal is really behind the posterior limit of the embryonic area which appears across the pointed pole of the egg. Ischikawa's account of the process of gastrulation in *Xiphocaridina* leaves many points obscure and a thorough comparison is difficult. He speaks of a band of cells on the side near one pole which becomes depressed and whose cells multiply and migrate into

the yolk. The depression is at first shallow but soon grows deeper, particularly in that half of the area which is nearer the pole. While this is going on a transverse elevation of cells is formed and this cuts the depression into two. That half of the band which is nearer the pole and which is more deeply depressed is, according to him, the late blastopore. The other half disappears. This description, so far as it goes, shows a large measure of agreement with my observations on *Caridina*. The late blastopore of Ischikawa would correspond to what has been called the definitive blastopore in the present account. The formation of this structure in *Caridina* is a more gradual and more elaborate process than in *Xiphocaridina*. The thick narrow band of cells at the end of which the definitive blastopore differentiates is not formed in *Xiphocaridina*. This band remains for a long time, even after the closure of the blastopore in *Caridina*, though the longitudinal groove which appears on it from one end to the other and which ultimately gives rise to the definitive blastopore, is short-lived.

In *Xiphocaridina* also the actual embryo is formed from a region lying some distance away from the late blastopore. But Ischikawa's account of these stages of development is so incomplete that a comparison is hardly possible. He mentions the formation of the anus somewhat in front of the late blastopore but does not say anything about the caudal furrow. Because of this Sollaud (1923) is positive that what Ischikawa describes as the anus is in reality the caudal furrow. It is possible that such a confusion has actually taken place (see Ischikawa's Fig. 62). Nevertheless it is clear enough from the description and the figures of Ischikawa that he did observe the formation of the embryo away from the blastopore. In *Caridina* there is a distinct gap between the region of the embryo surrounding the anus (here called the teloblastic area) and the definitive blastopore. The former represents the point where the two arms of the V-shaped band meet. It is on either side of this point and not of the blastopore that the teloblasts first appear. These observations are at variance with what is usually described for the Decapoda. Sollaud (1923) whose account is the most critical has shown that it is from the blastoporal area that the teloblasts are differentiated. In *Leander* there is no distinction between the blastoporal area and the teloblastic area. They are temporally separated but not spatially. The same is the case with *Eupagurus* (Krainska, 1936). In *Hemimysis* (Manton, 1928), *Nebalia* (Manton, 1934), *Mesopodopsis* (Nair, 1939) and *Squilla* (Nair, 1941) also—to cite only recent work on other groups—this distinction is not observed. While in all these forms the ectoderm of the embryo is organised immediately in front of and in direct continuity with the blastopore, *Caridina* (possibly also *Xiphocaridina*) shows a marked discontinuity. It is also

interesting to note that the patch of cells that is left at the blastoporal area remains unconnected with the body of the embryo for a long time.

There is no superficial differentiation of the germ layers in *Caridina*. Differentiation takes place as the cells migrate. The first cells that pass inwards absorb yolk and become the rudiments of the endoderm. This continues from the beginning of gastrulation till its very end. But mesoderm formation takes place only towards the end of gastrulation, *i.e.*, after the establishment of the definitive blastopore. Here, there is a marked difference from the observations of Sollaud (1923). He says that only one kind of cells migrate inwards from the early blastopore. Some of these get converted into vitellophags while the others remain close below the blastoporal area. This latter group of cells continues to give off vitallophags for some time and later, towards the end of gastrulation, organises itself into a compact cellular mass lying below the blastoporal area which Sollaud calls the "blastoporique bouchon". In addition to this two transverse bands of cells are formed, one on either side of the blastopore, which give rise to mesoderm cells along their inner aspect. These he calls the "mesectoblastic plates". It is these plates that give rise to the mesoderm of the naupliar segments.

The formation of mesoderm in *Caridina* is entirely different from this. Mesoderm cells arise from that side of the blastopore which is nearer the pointed pole of the egg. The cells pass in individually in the beginning. They pass below the surface layer of cells towards the pointed pole and then across it towards the front and gradually organise themselves into the head mesoderm bands which come to lie below the arms of the V-shaped band as the latter organises itself. The last formed mesoderm elements remain united together and move forwards in a body and take up a position below the teloblastic area. No indication of a " blastoporique bouchon " or of the " mesectoblastic plates " has been observed. The mass of last-formed mesoderm cells may perhaps be considered as corresponding to the " blastoporique bouchon," but its special features *vis-a-vis* the structure described by Sollaud should not be overlooked. The most important among these is the fact that it does not remain in the region of the blastopore, early or late. It moves away towards the front. Secondly it is decidedly mesodermal from the beginning. Sollaud's " blostoporique bouchon " is derived from cells which pass inwards from the early blastopore and which, in view of the fact that it continues to give off vitellophags, should properly be called mesendodermal. It is true that these cells unite to form the bouchon only after they have ceased to give off vitellophags, but in origin they are mesendodermal. This is not the case with *Caridina*. Here there is no mesendodermal complex. The two groups of germ elements are distinct from the moment of

their origin from the blastopore. It is also noteworthy that the early blastopore gives rise only to endoderm elements. In this total separation of the mesoderm and endoderm from the very beginning of gastrulation *Caridina* differs not only from the Palæmoninæ but also from *Eupagurus* (Krainska, 1936) *Nebalia* and *Squilla*. It shows greater resemblance to the Mysids in this respect though the absence of pregastral differentiation is an important difference from this group.

According to Sollaud the mesoderm of the head segments in the Palæmoninæ is formed by cells which sink in at the region of the mesectoblastic plates, independently of the blastopore, and wander forwards below the arms of the V-shaped band. The observations of the present author do not agree with this. In *Caridina* the naupliar mesoderm is blastoporal in origin and its development essentially similar to that of the Peracarida, *Nebalia* and *Squilla*. It may be pointed out here that in *Eupagurus* Krainska has described a similar origin for the head mesoderm. In this form gastrulation results in the formation of a mesendodermal mass from which the head mesoderm as well as the mesodermal teloblasts arise. She observes that the head mesoderm frequently degenerates, but considers it a pathological condition.

At the anterior end of the body two groups of mesoderm cells are formed independently of the blastopore. Their significance has already been touched upon. The posterior and more median pair of these mesodermal masses correspond to the preantennulary somite of *Hemimysis*, *Mesopodopsis*, *Nebalia* and *Squilla*. They become very massive in later development, divide into two blocks on each side, one forming an investment over the stomodæum together with its fellow of the opposite side, and the other descending into the labrum. Cœlomic sacs are differentiated in both these blocks that result from the preantennulary somite. The anterior pair of mesodermal masses (the pre-optic masses) do not appear to correspond to anything that has so far been described in any of the Crustacea. Neither is it possible to find any correspondence between these and the preantennary or labral somite that has been described by Roonwal (1938) in the African Migratory Locust, *Locusta*. Apart from the fact that the preoptic mesodermal masses in *Caridina* degenerate after a short time without undergoing any differentiation while the labral somite of *Locusta* has a protracted course of development, their points of origin are quite different. The former are more lateral than median, lying beyond the eye, while the latter is more median than lateral. It has also to be noted that the mesoderm of the labrum in *Caridina* is derived from the preantennulary mesodermal mass and not independently of it.

It has been stated that the mesoderm of the antennulary segment forms a pair of cœlomic sacs in later development. This corroborates the observation of Terao (1929) who has found the same condition in *Panulirus*.

In the Decapoda the teloblastic origin of the post-naupliar segments of the body has already been demonstrated in *Eupagurus* by Krainska (1936). The present work, therefore, provides the second instance which contradicts the contention of Sollaud that the post-cephalic segments alone have such an origin. The probable causes which led Sollaud to this inference have been mentioned in the foregoing pages. He takes cognizance of the ectodermal teloblasts only after they have formed a semicircular row and overlooks the fact that they not only pre-exist this condition but also undergo divisions. Krainska shows that they are first formed in two groups one on either side of the telson rudiment and only later become arranged in a semicircular row with a midventral teloblast. The mode of origin of the row of teloblasts is the same in *Caridina*, but there is this difference, that while in *Eupagurus* they begin to divide only after becoming arranged in a regular row, in *Caridina* the divisions start even before this. Despite this difference there is agreement between these two forms on the significant point that the teloblasts do form the last two cephalic segments also. This shows that there is, at least in so far as these two Decapods are concerned, a clear distinction between the naupliar and the post-naupliar regions of the body in respect of their origin. Adopting Sollaud's terminology the former is primary and the latter secondary. It is also significant that *Eupagurus* and *Caridina* belong to widely separated tribes under the Decapoda. This, possibly, shows that the distinction referred to above is widely prevalent among Decapoda and that, notwithstanding the findings of Sollaud, there is no difference as regards this fundamental point between the Eucarida and the other crustacea. It would appear that here too the nauplius though incipient, is clearly marked off from the succeeding stages, not merely by the earliness of its appearance but also by the intrinsic nature of its constituent tissues.

The development of the gut in *Caridina* is remarkable in several ways. All the three embryonic layers take part in its formation. Besides the stomodæum and the proctodæum which are ectodermal invaginations, it has a mesodermal as well as an endodermal component. The mesodermal part extends between the tip of the proctodæum and the boundary of the yolk mass at the base of the caudal papilla. The endodermal part formed from the yolk cells is the last to be organised and it is differentiated in the region of the yolk mass. The clear distinction between the endoderm and the mesoderm from the very beginning of gastrulation and the absence of a mesendodermal cell complex in *Caridina* have already been referred to.

It is, therefore, only reasonable to consider the cell mass that moves to a position below the teloblastic area at the end of gastrulation and which later comes to lie against the tip of the proctodæum as mesodermal in nature. After giving rise to the mesodermal teloblasts from its outer aspect this mass of cells organises itself into the rudiment of the gut. The yolk cells which are solely of blastoporal origin lie against its inner surface. As the caudal papilla grows, this mesodermal rudiment also grows by the active division of its component cells. There is here a marked difference from the growth of the gut rudiment in a form like *Squilla* (Nair, 1941), where it increases in extent by the addition of transformed yolk cells. This transformation is a very slow process particularly in the earlier stages and does not keep pace with the elongation of the caudal papilla. Consequently the rudiment gets stretched out and becomes thin and sparsely nucleated. It does not grow by division of the cells. In *Caridina*, on the other hand, the rudiment always appears thick and compactly nucleated. It does not show any indication of being stretched out and remains solid for a fairly long time. An epithelial endodermal plate appears against the proximal end of this mesodermal rudiment, but the two connect only at a later stage in development. Critical preparations which clearly show these important points have, fortunately, been obtained and their description can be found in the foregoing pages.

Sollaud (1923) has given a lengthy account of the development of the midgut in the Palæmoninæ. He has also critically examined the findings of earlier workers. Shorn of its elaborate verbiage his account indicates simply that the midgut has the same composition as obtains in *Caridina*. The proctodæum grows inwards to the base of the caudal papilla with the " blastoporique bouchon " attached to its tip. From the " bouchon ", which he considers as mesodermal, an intestinal plate is organised and this plate grows by the addition of modified yolk cells. In *Caridina* the place of the " blastoporique bouchon " is occupied by a mesodermal mass. It is this mass that grows and not the proctodæum which remains short and confined to the terminal part of the caudal papilla. The junction between the two can be clearly made out till a late stage in development. There is also this difference that while it may be said that the " blastoporique bouchon " is not purely mesodermal as it gives rise to yolk cells on the inside, in *Caridina* the gut rudiment that lies against the proctodæum consists of unmixed mesoderm as judged by its point of origin and properties. Manton (1934) has pointed out this difficulty in accepting the interpretation given by Sollaud and considers his conclusion regarding the mesodermal nature of the mid-gut rudiment as unjustifiable. I feel that this objection

cannot be raised against the observation on *Caridina* herein recorded. In this form the mesoderm forms a considerable length of the gut and not merely provides a small rudiment at the base of the caudal papilla as in *Leander*, granting that Sollaud's interpretation is correct. It may be pointed out in this connection that Sollaud also has shown that the gut inside the caudal papilla is formed as a solid strand of cells in the beginning and that a lumen is formed only subsequently. Terao (1929) has devoted some attention to the development of the gut inside the caudal papilla in *Panulirus* and he finds that it is mesodermal in origin.

The endodermal part of the gut is formed by the condensation of the yolk cells along the periphery of the yolk mass. The condensations assume the form of single layered epithelia. Three such epithelial rudiments are formed at the posterior side in front of the base of the caudal papilla; the middle one connects with the mesodermal gut rudiment in the caudal papilla and contributes to the formation of the midgut, while the two lateral ones give rise to the posterior liver lobes. Though all the three rudiments are truly endodermal it is noteworthy that they arise independently of one another and unite only later. It cannot, therefore, be said that the liver rudiments are formed as outgrowths from the gut primordium. In spite of this difference the origin of the liver in *Caridina* resembles that of the Palæmoninæ as described by Sollaud (1923). This is in marked contrast with the condition in the Mysids where the liver rudiments are mesodermal in origin (Manton, 1928; Nair, 1939). *Caridina* also shows evidence of the formation of two anterior liver lobes. These too are truly endodermal rudiments arising from independent condensations of the yolk cells on either side of the stomodæum. The findings of earlier workers on the origin and development of the liver in the Decapoda have been summarised and critically examined by Manton (1928 and 1934). The present work supports her view that the process of liver formation in the Decapoda is fundamentally different from that in the Peracarida.

Caridina hatches out in a relatively advanced stage of development possessing the first five pairs of abdominal appendages. In this it differs from the related form *Xiphocaridina* where Ischikawa (1885) observes no trace of the abdominal appendages in the newly hatched embryo.

Summary

1. The development of *Caridina lævis* Heller from the zygote to the newly hatched stage has been traced.

2. Hatching takes place 17 days after the beginning of cleavage.

3. Though the eggs are heavily yolked cleavage is total in the initial stages, resulting in the formation of equal, rounded blastomeres.

4. From the 16-blastomere stage the blastomeres progressively get rid of their burden of yolk with each division.

5. The non-nucleated yolk remains cut up into distinct blocks till a late stage.

6. The nucleated cytoplasmic islands of all the blastomeres ultimately condense on the surface to form the blastoderm.

7. Gastrulation starts from a point on the straight side of the egg.

8. The formation of yolk cells precedes that of the mesoderm cells which arise from one side of the definitive blastopore that is formed at the end of the blastoporal band.

9. There is no mesendodermal complex of immigrating cells, the endoderm and mesoderm elements being distinct from each other from the moment they sink in.

10. The proctodæum and the teloblastic area surrounding it are differentiated some distance away from the definitive blastopore.

11. Two paired groups of mesoderm cells arise independently of the blastopore from the anterior ends of the V-shaped band. The posterior group develops into the preantennulary mesoderm while the anterior group degenerates.

12. The first formed elements of the blastoporal mesoderm move forwards and give rise to the head mesoderm bands, while the last formed remain as a mass under the teloblastic area and give rise to the mesodermal teloblasts and to the rudiment of the midgut lying against the tip of the proctodæum.

13. The teloblasts give rise to all the post-naupliar segments of the body. There are 21 ectodermal teloblasts which arise in two groups on either side of the proctodæum and later arrange themselves in a continuous circular row.

14. Cœlomic sacs are developed in the preantennulary, antennulary and antennal somites.

15. Two blocks of mesoderm arising from the preantennulary somites, with cœlomic spaces in them, descend into the labrum.

16. The portion of the gut extending from the tip of the proctodæum to the base of the caudal papilla is formed by the growth of the mesodermal rudiment, as a solid rod which later becomes tubular.

17. The yolk cells give rise to a posterior endodermal plate at the base of the caudal papilla and to two pairs of liver lobes, one pair at the posterior end and the other at the anterior. The posterior endodermal plate later gets connected with the mesodermal gut inside the caudal papilla.

18. The newly hatched larva possesses the rudiments of the first five pairs of abdominal appendages.

References

(*Note.*—Only those papers which have been cited in the present account and those which have a direct bearing on the trend of the investigation are included in this list. Papers marked by an asterisk have not been referred to in original.)

*Bobretzky, N. .. "Sur l'embryologie des Arthropodes (Ecrevisse et *Palæmon*)," *Mem. Soc. Nat. Kiev.*, 1873, **3.**

*Brooks, W. K. .. "*Lucifer*: A Study in Morphology," *Phil. Trans. Roy. Soc. London*, 1882, **173.**

Bumpus, H. C. .. "The Embryology of the American Lobster," *Journ. Morph.*, 1891, **5.**

Calman, W. T. .. *A Treatise on Zoology, Part VII, Crustacea*, London, 1909.

Cannon, H. G. .. "Early Development of the Summer Eggs of a Cladoceran (*Simocephalus vetulus*)," *Quart. Journ. Micr. Sci.*, 1921, **65.**

———— .. "On the Development of an Estherid Crustacean," *Phil. Trans. Roy. Soc. London*, (B), 1924, **212.**

Faxon, W. .. "On the Development of *Palæmonetes vulgaris*," *Bull. Mus. Comp. Zool. Harvard*, 1879, **5.**

Gorham, F. P. .. "The Cleavage of the Egg of *Virbius zostericola*—A contribution to Crustacean Cytogeny," *Journ. Morph.*, 1895, **11.**

Ischikawa, Ch. .. "On the Development of the Fresh-water Macrurous Crustacean *Atyephira compressa* De Haan," *Quart. Journ. Micr. Sci.*, 1885, **25.**

*Kingsley, J. S. .. "The Development of *Crangon vulgaris*," *Bull. Essex Inst.*, 1887, **18.**

———— .. "The Development of *Crangon vulgaris*, Pt. 3," *ibid.*, 1889, **21.**

Korschelt, E., and Heider, K. *Text-Book of the Embryology of Invertebrates*, London, 1899, **3.**

Krainska, M. K. .. "On the Development of *Eupagurus prideauxi* Leach," *Extr. C. R. 12e Congr. Internat. Zool. Lisbonne* (1935), 1936.

MacBride, E. W. .. *Text-Book of Embryology, Vol.* 1, *Invertebrata*, London, 1914.

Manton, S. M. .. "On the Embryology of a Mysid Crustacean, *Hemimysis lamornæ*," *Phil. Trans. Roy. Soc.* (B), 1928, **216.**

———— .. "On the Embryology of the Crustacean *Nebalia bipes*," *ibid.*, 1934, **223.**

*Mayer, P. .. "Zur Entwicklungsgeschichte der Decapoden," *Zeit. f. wiss. Zool.*, 1877, **Bd. 29.**

Nair, K. B. .. "The Reproduction, Oogenesis and Development of *Mesopodopsis orientalis* Tatt.," *Proc. Ind. Acad. Sci.*, 1939, **9,**

———— .. "On the Embryology of *Squilla*," *ibid.*, 1941, **14,**

*Reichenbach, H. .. "Studies zur Entwicklungsgeschichte des Fluskresbses," *Abh. Senckenb. Ges. Frankfurt*, 1886, **14.**

*———— .. "Die Embryonalanlage und erste Entwicklung des Fluskrebses," *Zeit. f. wiss. Zool.*, 1887, **Bd. 29.**

Roonwal, M. L. .. "Studies on the Embryology of the African Migratory Locust, *Locusta migratoria migratorioides*, Pt. I," *Phil. Trans. Roy. Soc. London*, 1936, **226.**

———— .. Do., Part II, *ibid.*, 1937, **227.**

———— .. "Some Recent Advances in Insect Embryology with a Complete Bibliography of the Subject," *Jour. Roy. Asiatic Soc., Bengal*, 1938, **4.**

———— .. "Amplification of the Theory of Multi-phased Gastrulation among Insects, and its applicability to some other Anthropods," *Trans. Nat. Inst. Sci. India*, 1939, **2.**

Sollaud, E. .. "Recherches sur L'Embryogenie des Crustaces Decapodes de la sous-famille des Palemoninæ," *Bull. Biol. France et Belg. Suppl.*, 1923, **5.**

Terao, A. .. "The Development of the Spiny Lobster *Panulirus*," *Jap. Journ. Zool.*, 1929, **2.**

Von Bonde, C. .. "The Reproduction, Embryology and Metamorphosis of the Cape Crawfish (*Jasus lalandi*)," *Mar. Biol. Repts. South Africa*, 1936, **6.**

Welden, W. F. R. .. "The Formation of the Germ layers in *Crangon vulgaris*," *Quart. Journ. Micr. Sci.*, 1892, **33.**

Yonge, C. M. .. "The Nature and Significance of the Membranes surrounding the Developing Eggs of *Homarus vulgaris*," *Proc. Zool. Soc. London*, Series A, 1938, **107.**

Zehnder, H. .. "Uber die Embryonalentwicklung des Fluskrebses," *Acta Zoologica, Stockholm*, 1934, **15.**

Explanation of Figures

(*Note*.—The magnifications given refer to the originals of the figures which have been photographically reduced in preparing the blocks.)

Fig. 1. Longitudinal section of the zygote. ×130.
Fig. 2. Enlarged view of the periphery of the zygote in section. ×1,600.
Fig. 3. The completion of the first cleavage. ×100.
Fig. 4. The second cleavage. ×100.
Fig. 5. The four-blastomere stage. ×100.
Fig. 6. The eight-blastomere stage. ×100.
Fig. 7. The sixteen-blastomere stage. ×100.
Fig. 8. Stage with approximately 64 blastomeres. ×100.
Fig. 9. Stage 28 hours after the beginning of cleavage. ×100.
Fig. 10. Stage 36 hours after the beginning of cleavage. Sketched from fixed material. ×100.
Fig. 11. T.S. of the embryo at the 16-blastomere stage. ×130.
Fig. 12. L.S. of the embryo at the 16-blastomere stage. ×130.
Fig. 13. The cleavage spindle at the 16-blastomere stage. ×800.
Fig. 14. The cleavage nucleus at the 16-blastomere stage. ×800.

Fig. 15. Section showing the beginning of gastrulation. Stage very near that represented in Fig. 10. ×1,600.

Fig. 16. Surface view of the fixed embryo showing the blastoporal band. ×100.

Fig. 17. T.S. of the embryo showing the longitudinal depression in the blastoporal band. ×130.

Fig. 18. L.S. of the embryo approximately 48 hours after the beginning of cleavage showing the blastoporal band and the definitive blastopore. ×100.

Fig. 19. T.S. of the blastoporal band. ×800.

Fig. 20. T.S. of the blastoporal band with the longitudinal depression which is deepening to form the definitive blastopore. ×800.

Fig. 21. L.S. showing the definitive blastopore and the origin of the mesoderm. The lower side of the figure is towards the pointed pole of the egg. ×800.

Fig. 22. L.S. passing through the region of the definitive blastopore as it is just closing. ×330.

Fig. 23. L.S. of the embryo at a stage immediately after the closing of the definitive blastopore. The V-shaped band is just getting organised. The section being median sagittal cuts the band only across a limited area. ×100.

Fig. 24. Surface view of the embryo (fixed) showing the V-shaped band on one side. Stage 50 hours after the beginning of cleavage. ×100.

Fig. 25. Parasagittal section of the embryo belonging to the same stage as the one represented in Fig. 24, showing the teloblastic area and the V-shaped band. ×180.

Fig. 26. Ventral view of the embryo (fixed) showing the naupliar region. Stage approximately 60 hours after the beginning of cleavage. ×130.

Fig. 27. Side view of the embryo shown in Fig. 26. ×130.

Fig. 28. Diagram of a whole mount of the teloblastic area (about 60 hours from the beginning of cleavage) showing the organisation of the ectodermal teloblasts. ×800.

Fig. 29. L.S. passing through the anterior region of the V-shaped band showing the preoptic mesodermal rudiment about to sink in. ×1,600.

Fig. 30. L.S. of the V-shaped band showing the prenatennulary mesoderm rudiment about to sink in. ×1,600.

Fig. 31. Parasagittal section passing through the teloblastic area and one arm of the V-shaped band showing the first indication of the caudal furrow. ×280.

Fig. 32. L.S. of the V-shaped band showing the head mesoderm band. ×280.

Fig. 33. Diagram of a whole mount of the telobalstic area (about 72 hours from the beginning of cleavage) showing the caudal furrow, ectodermal teloblasts and the proctodæum. ×800.

Fig. 34. Median sagittal section of the nauplius stage showing the stomodæum and the group of mesodermal cells below the teloblastic area. ×650.

Fig. 35. Parasagittal section of the nauplius stage showing the posterior end of the head mesoderm band and the caudal furrow. ×650.

Fig. 36. T.S. of the teloblastic area showing the proctodæal rudiment and the mesodermal plate lying against its tip. The ectodermal as well as the mesodermal teloblasts could be seen. ×800.

Fig. 37 A. Outline of the late nauplius stage. ×220.

Fig. 37 B. Surface view of the same, enlarged. ×800.

Fig. 38. Median sagittal section of the late nauplius stage showing the boundary between the teloblastic area and the mandibular segment. The median ectodermal teloblast is seen in the process of division. ×800.

Fig. 39. Parasagittal section of the same stage as represented in Fig. 38 showing the limit of the mandibular segment and the position of the ectodermal teloblasts. ×800.

Fig. 40. Parasagittal section of a slightly earlier stage passing through the same region as that shown in Fig. 39. ×800.

Fig. 41. Sagittal section passing through the region of the optic rudiment of the late nauplius stage. The pre-optic mesoderm rudiment is undergoing degeneration. ×520.

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Fig. 43. Side view of the embryo 60 hours from the beginning of cleavage. ×170.

Fig. 44. Embryo 102 hours from the beginning of cleavage. Side view. ×170.

Fig. 45. Side view of the embryo 126 hours from the beginning of cleavage. ×330.

Fig. 46. Side view of the embryo 150 hours from the beginning of cleavage. ×220.

Fig. 47. Side view of the embryo 9 days old. ×220.

Fig. 48. Side view of the embryo 12½ days old. ×220.

Fig. 49. Dorsal view of the embryo 12½ days old. ×220.

Fig. 50. Embryo 16 days old torn out of the envelope. ×70.

Fig. 51. The newly hatched larva. ×40.

Fig. 52. Anterior end of the newly hatched larva. Dorsal view. ×130.

Fig. 53. Posterior end of the newly hatched larva. Dorsal view. ×130.

Fig. 54. T.S. passing through the region of the caudal papilla of a stage corresponding to that shown in Fig. 43 showing the proctodæum, mesodermal plate and the ectodermal and mesodermal teloblasts. ×650.

Fig. 55. T.S. of the same stage as above passing through the mandibular segment. ×650.

Figs. 56–58. Transverse sections passing through the antennal segment of the same stage as shown in Fig. 43, in order from behind forwards showing the mesodermal blocks ×650.

Figs. 59 & 60. Two transverse sections of the same satge as shown in Fig. 43 passing through the antennulary segment showing the mesoderm block and the cœlomic cavity therein. ×650.

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Fig. 64. T.S. passing through the first thoracic segment showing the mesodermal somite and the coelomic sac. Stage same as that shown in Fig. 63. Here also the endodermal layer is visible. ×800.

Fig. 65. T.S. of the caudal papilla at a point not far from the tip. Stage same as that shown in Fig. 46. ×1,600.

Fig. 66. T.S. of the caudal papilla showing the further differentiation of the mesoderm. ×1,100.

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Fig. 80. T.S. of the caudal papilla of a late stage showing the pericardial floor. ×800.

Fig. 81. Slightly parasagittal section of the anterior end of a late embryo showing the protocerebrum, antennulary ganglion and the antennal ganglion. ×520.

Fig. 82. T.S. of the stomodæum of a late stage showing the preantennulary mesodermal investment and the cœlomic space that is developed all round. Differentiation of the mesoderm into muscles has progressed considerably. ×800.

Fig. 83. T.S. of the gut and the pericardial floor of an advanced stage showing the paired rudiment of the gonad. ×800.

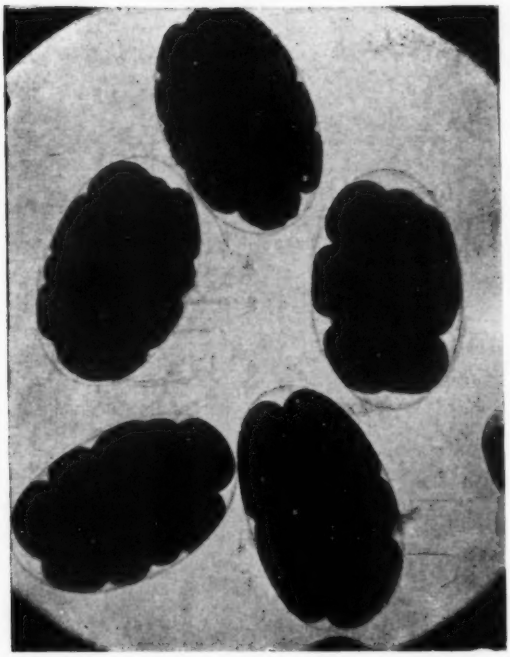
Fig. 84. Median sagittal section of an embryo of a stage corresponding to the one represented in Fig. 48. ×170.

Fig. 85. Parasagittal section of the same stage as shown in Figs. 84. ×170.

List of Abbreviations

A .. straight side.
a.li.ru. .. rudiment of the anterior liver lobe.
ant. .. antenna.
ant.g. .. antennal ganglion.
antl. .. antennule.
antl.g. .. antennulary ganglion.
B .. curved side.
bl. .. blastomere.
blp. .. blastopore.
blp.b. .. blastoporal band.
br. .. brain.
C .. pointed pole.
c. .. blood corpuscle.
cd.f. .. caudal furrow.
cd.p. .. caudal papilla.
cl.f. .. cleavage furrow.
coel.s. .. cœlomic space.
com. .. circum-œsophageal nerve commissure.
crp. .. carapace.
cyt. .. cells constituting the blastoderm.
D .. blunt pole.

d.blp. .. definitive blastopore.
dbv. .. dorsal blood vessel.
dlb. .. dorso-lateral block of mesoderm.
ect. .. ectoderm.
ect.tel. .. ectodermal teloblasts.
end. .. endodermal layer.
end.g. .. endodermal gut.
end.pl. .. endodermal plate.
env. .. ovarian envelope.
f. .. filament attaching the egg to the pleopod.
g. .. gut.
gl.ru. .. rudiment of the gills.
g.m. .. mesodermal investment of the gut.
go.ru. .. rudiment of the gonad.
h.mes.b. .. head mesoderm band.
ht. .. heart.
is. .. interspace between the blastomeres.
lb.pm. .. labral blocks of the preantennulary mesoderm.
lbr. .. labrum.
li.ru. .. posterior liver rudiment.
mb. .. middle block of mesoderm.
mdb. .. mandible.
m.g. .. mesodermal gut.
mes. .. mesoderm.
mes.c. .. mesoderm cell.
mes.gru. .. mesodermal gut rudiment.
mes.pl. .. mesodermal plate.
mes.tel. .. mesodermal teloblasts.
mes.tel.d. descendants of the mesodermal teloblasts.
mus. .. muscle.
mx. 1 .. first maxilla.
mx. 2 .. second maxilla.
n.c. .. nerve cord.
n.g. .. nerve ganglion.
nu. .. nucleus.
opt. .. eye.
opt.ru. .. optic rudiment.
p.a.m. .. preantennulary mesoderm.
pf. .. pericardial floor.
plp. .. pleopods.
p.op.m. .. preoptic mesoderm.
prc. .. protocerebrum.
prct. .. proctodæum.
r. .. rostrum.
spl. .. division spindle.
stm. .. stomodæum.
st.pm. .. stomodæal block of the preantennulary mesoderm.
t. .. telson.
tel.a. .. teloblastic area.
th. 1 .. first thoracic appendage.
th. 2 .. second thoracic appendage.
th. 3 .. third thoracic appendage.
t.r. .. telson rudiment.
v.b. .. V-shaped band.
vbm. .. ventral block of mesoderm.
y. .. yolk.
y.c. .. yolk cell.



A group of eggs undergoing cleavage. One egg is in the 8-blastomere stage. The others are in the 16-blastomere stage. ×40.

STUDIES IN THE GENUS COLLETOTRICHUM

Part IV. Leaf Spot of *Arundo donax* L. Caused by *Colletotrichum falcatum* Went. var. *arundinis* var. nov.

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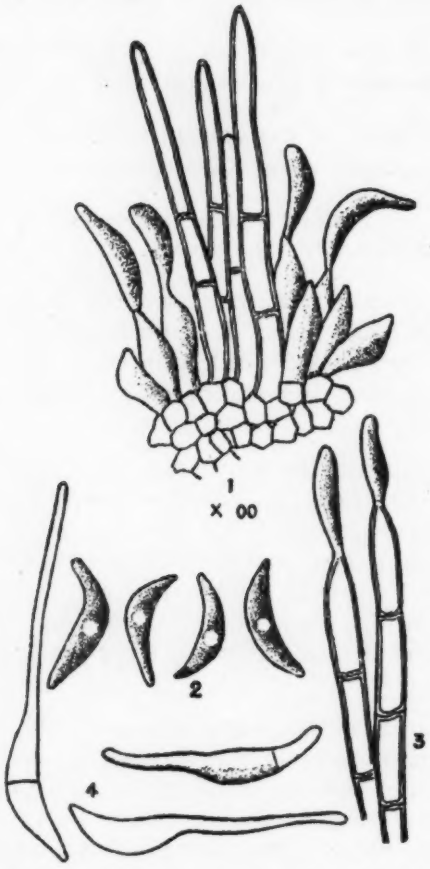
Arundo donax is a common reed grass growing on the banks of rivers, streams and tanks in South India. It is usually planted round pan (*Piper betle* L.) gardens to form thick live hedges for protection. A leaf spot-cum-stripe disease of this plant is prevalent round about Coimbatore almost throughout the year.

The disease is apparent on most of the leaves excepting the youngest ones at the tips of the shoots. Elongated or spindle-shaped spots develop on the leaves. These are isolated in the initial stages but very often coalescence of the spots takes place and large patches result. Individual spots may measure up to 2×1 cm. Mature spots are straw yellow in colour with a thin brown margin. Beyond the spot is a halo of yellowish green colour. The tissues of the leaf easily tear along the length in the spotted region.

On the upper surface of the spots grooves are visible between the veins. In this region the epidermis easily gives way and numerous erumpent dark brown linearly arranged acervuli are clearly recognisable. The epidermis on the lower surface of the spots however remains intact and acervuli are rare or not formed on this surface. Each acervulus has a subepidermal light to dark brown stroma. The large number of acervuli formed subepidermally in the interveinal region causes the rupture of the epidermis in lines between the veins. From the upper portion of the stroma setæ and conidiophores are produced. The setæ are brown, septate with one to four septa, pointed or blunt and measure $73 \times 6\,\mu$ (48–117 × 4–7). Rarely, conidia develop from the tips of the setæ also. The conidiophores are non-septate, hyaline to sybhyaline and mixed with the setæ. Conidia are hyaline, one-celled, falcate, broadest in the middle and tapering towards the ends either equally or unequally. Fresh spores have granular contents with a prominent

central vacuole but in older spores more extensive vacuolation is evident. The spores measure 29 × 6 μ (18–39 × 3–7).



TEXT-FIG. 1. Acervulus. 2. Conidia. 3. Setæ bearing conidia. 4. Germination (×500).

The morphological features of the fungus are akin to those of *Colletotrichum graminicolum* (Ces.) G. W. Wils. and *Physalospora tucumanensis* Speg. (*C. falcatum* Went.), two other species common on gramineaceous hosts in the locality. The conidia of all the three are almost similar though the symptoms of infection on the respective hosts are quite different. Comparative measurements of 200 spores each of *C. graminicolum*, *C. falcatum* and the fungus under study taken from their respective hosts, were made; the measurements are recorded hereunder.

TABLE I

Measurements of conidia of the three fungi

Isolate	Length		Breadth	
	Range in μ	Average μ	Range in μ	Average μ
C. graminicolum ..	21—32	26·5	3—7	5·6
C. falcatum ..	21—39	28·0	3—7	5·0
C. on *Arundo* ..	19—39	29·5	3—7	5·7

The measurements exhibit close approximation excepting that the conidia of *C. graminicolum* have a shorter range in length and a slight reduction in the mean length than those of the others.

The fungus grows very slowly on oat and french bean agar media. The submerged growth consists of coloured septate hyphæ imparting an olive black colour to the growth. The aerial growth is scanty and is made up of hyaline hyphæ resulting in a white or grey loose cottony mycelium. Numerous olive brown appressoria (chlamydospores) of varying shapes develop on the sides of the tubes and sometimes submerged in the media. Conidia are formed by the aerial mycelium. These develop in clusters but the characteristic pink coloured masses usually seen in the initial cultures of *C. falcatum* and *C. graminicolum* are not produced by this fungus. The slow rate of growth of this isolate can be readily seen from the growth measurements of the three fungi made six days after inoculation on three different agar media. The measurements are recorded below and represent the average of triplicate cultures (Plate XIII, Fig. 2).

TABLE II

Growth measurements of C. falcatum, C. graminicolum *and the isolate from* Arundo donax *after 6 days*

	Diameters of growths on		
	Richards agar mm.	Brown's agar mm.	French bean agar mm.
C. Graminicolum ..	42·3	34·0	58·0
C. falcatum ..	44·0	39·5	67·0
C. on *Arundo* ..	5·5	7·0	8·5

The growths of *C. falcatum* and *C. graminicolum* are more extensive and consist of dark grey luxuriant aerial growth. In marked contrast with this the growth of the isolate from *Arundo* is limited and produces only scanty aerial growth. Further the cultures of this isolate are not amenable to continuous subculturing as they fade out in the course of a year. Frequent fresh isolations are thus needed to keep the culture going.

Inoculation experiments.—The pathogenicity of the isolate from *Arundo donax* was tested on different host plants—*Arundo donax*, sugarcane and sorghum. Suspensions of the conidia of the isolate were sprayed on the leaves of young plants grown in pots and kept inside glazed cages under humid conditions. Formation of lesions became evident in 3 days on young leaves of *Arundo donax* and in 6 days on older leaves. Acervuli developed in 10–15 days and the same fungus was reisolated from the infected plants. On sorghum and sugarcane leaves however there was no infection. The experiments were repeated on the leaves of the latter by placing drops of spore suspension on wounds caused by needle punctures. Small reddish spots developed in a week on sorghum but these spots were of a uniform colour and did not exhibit the characteristic appearance of the anthracnose spots on sorghum. Wound inoculation was successful on the mid ribs of sugarcane leaves.

Infection experiments were conducted on the stem of growing sugarcane plants. Holes were made with sterilised cork-borer in the third internode from the ground. Bits of the culture were introduced into these holes after which the latter were plugged. Melted paraffin was smeared over the surface. Three weeks later the canes were cut and examined. It was found that the inner tissues were reddened above and below the point of inoculation and the reddening extended to the next internode. The fungus was reisolated from the reddened tissue. The controls did not exhibit any extension of reddening beyond the bore hole.

Suspensions of the conidia of *C. falcatum* and *C. graminicolum* sprayed on the leaves of *Arundo donax* did not produce any symptoms of infection even after 3 weeks. The conidia of *C. falcatum* and *C. graminicolum* were used for inoculating the leaves of sorghum. *C. graminicolum* produced typical anthracnose spots with whitish centre and dark red margin in 5–7 days. Black dots representing acervuli developed in the centre of the spots in the course of 10 days. *C. falcatum* however did not infect the leaves unless they were previously wounded. Even then the spots that developed were of uniform red colour as those formed in infections by *Colletotrichum* from *Arundo donax*.

C. graminicolum has been recorded on several grasses (Wilson, 1914). Though some of the earlier records of this fungus on the different hosts were under different names, Wilson considered them to be identical, having a range of conidial measurements of 18 to 26 × 3 to 6 μ with one or two forms exhibiting a range of 20 to 30 × 4 to 7 μ. Dickson (1947) has mentioned the occurrence of *C. graminicolum* on several grasses and cereals and has stated that the fungus causes rot of root, crown and culm of the grass hosts in addition to the formation of spots on sorghum. Abbott (1938) has collected different isolates of *C. falcatum* from *Sorghum sudanense*, *Sorghum halepense* and *Erianthus* sp. These are according to him morphologically indistinguishable from the races of *C. falcatum* on sugarcane. The range of measurements of the conidia was 16–48 × 4–8 μ.

The fungus under study exhibits closer affinity to *C. falcatum* in spore length than to *C. graminicolum*. Further the results of infection on sorghum leaves by this fungus are similar to those of *C. falcatum* and different from those of *C. graminicolum*. The isolate from *Arundo* is also capable of infecting standing sugarcane stem. But it differs from *C. falcatum* in the extremely slow growth and absence of pinkish spore masses in cultures. For these reasons it is identified as a variety of *C. falcatum* and named *C. falcatum* Went var. *arundinis*. This fungus is common on *Arundo donax*. The host plant is often seen in the vicinity of sugarcane fields. The fungus can infect wounded leaves and stem of sugarcane more or less in a manner akin to that of *C. falcatum*. Thus it is a source of potential danger. It is advisable to eradicate this host whenever it grows near sugarcane fields in order to avoid the danger of infection to sugarcane.

C. falcatum var. *arundinis*. Spots amphigenous more prominent on the upper surface, spindle shaped, isolated or confluent; acervulus epiphyllous small subepidermal, erumpent, in lines; setæ brown, septate, 73 × 6 μ (48–117 × 4–7); conidium hyaline falcate, one-celled 29 × 6 μ (18–39 × 3–7).

On living leaves of *Arundo donax* L. Coimbatore 10–6–42 T. S. Ramakrishnan.

Maculæ amphigenæ, extanter epiphyllæ, fusiformæ, isolatæ vel confluentæ; acervulis epiphyllis, subepidermalibus, erumpentis, minutis, seratim in lineas dispositus; setæ brunneæ, septatæ, 73 × 6 μ (48–117 × 4–7); coniidis hyalinis, falcatis, unicellatis 29 × 6 μ (18–39 × 3–7).

In vivis foliis *Arundinis donacis* L., Coimbatore 10–6–42 T. S. Ramakrishnan.

Summary

A leaf spot disease of *Arundo donax* is prevalent in Coimbatore. This is caused by *Colletotrichum*. The pathogen was isolated and compared with *C. graminicolum* and *C. falcatum*. It is capable of infecting sugarcane. The characters of the fungus show its similarity to *C. falcatum* but it differs from the latter in its poor growth. It is described as a variety of *C. falcatum*.

Literature Cited

Abbott, E. V. .. *U. S. Dept. Agri. Tech. Bull.*, 1938, 641.

Dickson, J. G. .. *Diseases of Field Crops*, McGraw Hill Book Co., London, 1947, 182–97 and 255–89.

Wilson, G. W. .. *Phytopath*, 1914, 4, 106–13.

Explanation of Plate

1. Leaves of *Arundo donax* showing lesions (reduced).
2. Cultures of *C. graminicolum* (*c. g.*), *C. falcatum* (*c. f.*) and *C.* on *Arundo donax* (*C.a.*) on Richards (*R*), Brown's (*B*) and French bean (*F*) agars.

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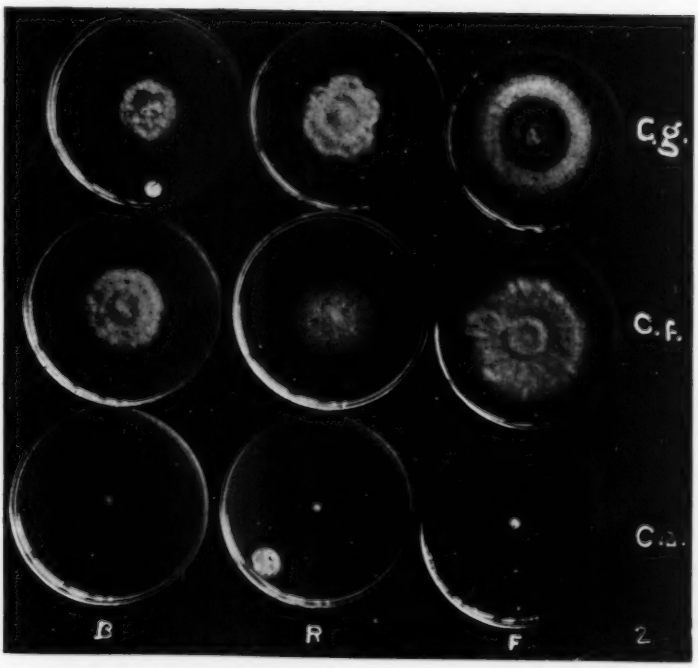
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C.F.
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